GENETICS

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BIOLOGICAL ABSTRACTS
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INHERITANCE OF RESISTANCE TO MILDEW, ERYSIPHE GRAMINIS HORDEI, IN THE BARLEY VARIETY, BLACK RUSSIAN

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Received September 7, 1954

SINCE BIFFEN (1905) first established that resistance to disease-inciting organisms in plants followed Mendelian laws, the entire field of host-pathogen relationships has been subjected to extensive investigations. Numerous among them have been those pertaining to the genetic mechanism of disease resistance. While notable contributions have been made in this field, few attempts have been made to explore the total genetic potential (in terms of resistance) of a host species to a specific pathogen. However, the results of FLOR's work with flax rust, *Melampsora lini* (Pers.) Liv. (FLOR 1947, 1954), and that of BRIGGS and associates (BRIGGS and STANFORD 1938; STANFORD and BRIGGS 1940; FAVRET 1949) with powdery mildew, *Erysiphe graminis hordei* (DC.) Marchal, of barley suggest that the genetic variation in terms of resistance within certain species to specific pathogens is essentially unlimited. At least 22 genes conditioning resistance to flax rust and 9 genes responsible for resistance to a single race of powdery mildew of barley have been identified. In both cases, only a small sample of the total germ plasm has been examined.

The genetics of resistance to race 3 of powdery mildew in 12 varieties of barley has been determined (BRIGGS and STANFORD 1938; STANFORD and BRIGGS 1940; FAVRET 1949). Nine genes, 7 dominant or incompletely dominant and 2 recessive, have been identified. The resistance of 8 varieties was conditioned by a single gene pair, by duplicate gene pairs in 3 varieties and by 3 gene pairs in one variety. The 12 varieties, together with the genes responsible for their resistance are listed below:

Variety	Genes conferring resistance	Variety	Genes conferring resistance
Hanna	Ml_hMl_h	West China	ml_wml_w
Goldfoil	Ml_gMl_g	Psaknon	Ml_rMl_p
Algerian	Ml_aMl_a	Chinerme	Ml_pMl_p; Ml_yMl_y
S.P.I. 45492	Ml_aMl_a	Nigrate	Ml_pMl_p; Ml_yMl_y
Kwan	Ml_kMl_k	Arlington Awnless	Ml_pMl_p; Ml_yMl_y
Monte Cristo	Ml_mMl_m	Duplex	Ml_pMl_p; Ml_hMl_h; ml_dml_d

Linkage between the Ml_a and Ml_k and the Ml_p and ml_d loci has been established (BRIGGS and STANFORD 1938; BRIGGS 1945).

FAVRET (1949) identified two additional genes, Ml_c and ml_n, which impart resistance to races A-1 and A-2, but which are susceptible to race 3. While other single gene segregations have been reported in crosses between resistant and susceptible varieties, their relationships to the genes discussed above is not known. However, the gene identified in the variety Nepal (TIDD 1940) which gives protection against

races 6 and 7 must be different since Nepal is susceptible to race 3. Thus, at least 12 genes are known which confer resistance to the various physiologic races of powdery mildew.

In this paper another gene for mildew resistance which forms an allelic series with one previously identified is discussed. Additional information on the linkage relationships of the various genes is presented.

MATERIALS AND METHODS

The variety Black Russian, C.I. 2202, was found to be resistant to mildew by DR. G. A. WIEBE (private correspondence) in the field at Madison, Wisconsin. While resistant to race 3 in California, its level of resistance is not as high as that possessed by a number of other varieties. Black Russian is a head selection from C.I. 705, an introduction from the Caucasian region of Russia. Except as parental material, it has no commercial value in the United States.

Black Russian was crossed with the susceptible variety Atlas and with the resistant varieties Hanna, Goldfoil, Psaknon, Algerian, Chinerme, Kwan and Selection 175, which are testers for seven of the genes discussed previously. Segregation was studied in the F_2 generation and, in pertinent crosses, confirmed in the F_3 generation.

The hybrid populations, together with their respective parents, were grown in greenhouse benches. Susceptible Atlas checks were included every twentieth row. The plants were inoculated at the three-leaf stage with race 3 of mildew by dusting the spores over the plants from infected plants grown for this purpose. The plants were classified according to the system suggested by MAINS and DIETZ (1930) as follows:

Type 0—Highly resistant, no mycelium evident. Chlorotic or necrotic spots may be developed by some varieties.

Type 1—Very resistant, slight to moderate mycelial development, but with little or no sporulation. Chlorotic or necrotic spots may develop in some varieties.

Type 2—Moderately resistant, moderate mycelial development, accompanied by limited sporulation. Chlorotic or necrotic areas may be formed.

Type 3—Moderately susceptible. Moderate to abundant mycelial development, accompanied by moderate sporulation.

Type 4—Very susceptible. Abundant mycelial development, accompanied by abundant sporulation.

A high level of infection was obtained throughout these tests; the susceptible checks were consistently classified as type 4. The resistant parent, Black Russian generally gave a type 2 reaction, although under certain conditions, which will be discussed later, it was classified as types 0 or 1. In previous investigations at this station no attempt was made to differentiate between types 3 and 4. Phenotypically the plants were either resistant (types 0, 1 and 2) or very heavily mildewed (type 4). Plants were encountered in crosses involving Black Russian which were intermediate between types 2 and 4 and were thus classified as type 3. This was the first indication that the genetic factors responsible for resistance in Black Russian differed from those previously identified.

TABLE 1

The classification of the parents and F_2 plants or F_3 rows, as indicated, of the crosses named and goodness of fit to expected ratios based on independent segregation

Generation	Parent or hybrid	Observed numbers			Ratio	Value of P for ratios indicated
		Resistant	Heterozygous rows or type 3 plants	Susceptible		
	Atlas*			550		
	Black Russian*	510				
F_2	Atlas × Bl. Russian†	155	268	130	1:2:1	>0.20
F_2	Atlas × Bl. Russian‡	129	69	59	1:2:1	<0.01
F_2	Atlas × Bl. Russian§	621		189	3:1	>0.20
	Atlas (rows)			6		
	Bl. Russian (rows)	6				
F_3	Atlas × Bl. Russian (rows)	32	64	28	1:2:1	>0.80
	Hanna (Ml_h)	72				
F_2	Bl. Russian × Hanna	205		17	15:1	>0.30
	Goldfoil (Ml_g)	60				
F_2	Bl. Russian × Goldfoil	197		13	15:1	>0.90
	Chinerme (Ml_p; Ml_y)	55				
F_2	Bl. Russian × Chinerme	369		3	63:1	>0.30
	Psaknon (Ml_p)	150				
F_2	Bl. Russian × Psaknon	1268		57	15:1	<0.01
	Selection 175 (ml_a)	85				
F_2	Bl. Russian × Sel. 175	695		64	13:3	<0.01
	Kwan (Ml_k)	75				
F_2	Bl. Russian × Kwan	617		2	15:1	<0.01
	Kwan (rows)	4				
F_3	Bl. Russian × Kwan (rows)	58	6	0	7:8:1	<0.01
	Algerian (Ml_a)	240				
F_2	Bl. Russian × Algerian	4300		0	15:1	<0.01
	Algerian (rows)	15				
F_3	Bl. Russian × Algerian (rows)	300	0	0	1:2:1	<0.01

* Atlas and Black Russian grown at 20 row intervals throughout all tests.

† Series I—Heterozygous plants positively identified (type 3).

‡ Series II—Heterozygous plants not positively identified (see text).

§ Resistant and type 3 plants combined (Series I and II).

EXPERIMENTAL RESULTS

The segregation obtained in the F_2 and F_3 generations of the various crosses is given in table 1. In the F_2 population of Black Russian × Atlas there were 621 resistant to 189 susceptible plants, which is in agreement with the 3:1 ratio expected for monofactorial segregation (P = 0.20 − 0.10). In the F_3 generation there were 32 resistant, 64 segregating and 28 susceptible families, which is in good agreement with the expected 1:2:1 ratio (P = 0.95 − 0.80) for a single gene difference.

It was soon apparent that the reaction of the resistant parent as well as the heterozygote varied with the environmental conditions under which it was tested. Under

certain conditions considerable mycelial development and sporulation, accompanied by necrotic spotting, was evident on the Black Russina parent (type 2). Under these conditions the heterozygotes showed considerable sporulation (type 3) and could be distinguished phenotypically from the homozygous genotypes, permitting complete classification. The F_2 population tested under these conditions segregated in the ratio of 1 type 2: 2 type 3: 1 type 4 (table 1). In other tests the resistant parent was completely free from mycelial growth (type 0) or showed only occasional flecking (type 1). In these tests the heterozygotes varied from types 1 to 2, and could not be separated phenotypically from the homozygous resistant combination (table 1). That this variation in reaction resulted from an interaction with environmental factors rather than from a variation in infection levels was shown by the consistently high infection on the susceptible progenies and checks. Thus gene expression and the degree of dominance were markedly affected by the environmental conditions.

Although no attempt was made to determine the environmental factors responsible for this variation in gene expression, the level of resistance was always the highest in tests made during February and March and lowest in December and January. While temperatures averaged slightly higher during the latter part of the winter, the greatest differences appeared to be in light intensity and in day length. Consequently, these two factors might be the main cause of the variation.

Segregation for susceptible types occurred in all test crosses, except with Algerian which possesses the Ml_a gene. Agreement between the observed and expected distributions for independent segregation was good for the crosses involving Hanna, Goldfoil and Chinerme, with P values ranging from 0.2 to 0.98 (table 1). Association was suggested in crosses with Kwan (Ml_k), Psaknon (Ml_p), and Selection 175 (ml_d) with P values for independent segregation smaller than 0.01 (table 1).

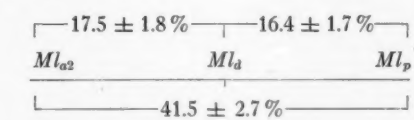
Since no segregation occurred in crosses with Algerian, the gene for resistance in Black Russian is either the same as the Ml_a gene carried by Algerian, closely linked, or an allele of it. Phenotypically, however, the genes could be readily distinguished. The Ml_a gene in Algerian produced a type 0 reaction and was completely dominant in expression. The gene conditioning resistance in Black Russian permitted a type 2 infection when homozygous and under certain environmental conditions, a type 3 reaction when heterozygous. Segregates were readily classified into the respective parental types. If linkage is assumed, the two genes would enter the cross in the repulsion phase. A susceptible type, the double recessive, would result from the union of 2 crossover gametes, the single heterozygotes from the union of a crossover gamete with a parental gamete. Neither type was identified in the F_2 populations or by F_3 progeny tests, suggesting that if recombination occurred, the percentage was extremely small. The combined estimate of linkage by the method of maximum likelihood (ALLARD 1955) from the F_2 and F_3 data, gave a recombination value of 0.00 ± 1.27 percent, indicating that the true recombination value falls somewhere within the range of 0.00 to 2.54 percent. However, since a test of this kind is not too sensitive, further information on the recombination value can be obtained by examination of the populations on which this estimate was based. In the F_2 population of 4300 individuals (table 1) at least one susceptible plant would have been expected ($P = 0.95$) if the recombination value was 5.29 percent or

greater. One thousand nine hundred and eighty plants of the above F_2 population were tested under conditions, as discussed previously, which permitted the positive identification of the single heterozygote of the Black Russian gene. In a population of this size at least one such plant would have been expected (P = .95) with a recombination value of 0.30 percent or greater. In neither of the populations were plants of these types observed.

Progenies from 300 random F_2 plants were tested for their reaction to mildew. Recombination products giving rise to the single heterozygotes could be readily identified by segregation ratios of 3 resistant:1 susceptible plant. Positive family classification (P = .999) was assured by populations of 25 or more plants. In a population of 300 F_3 families, at least one such segregating family would have been expected (P = .95) if the recombination value was greater than 1.0 percent. None were found. The F_3 families fell into 3 groups, those homozygous for the parental reactions and those segregating for the parental reactions, in the following ratio: 78 Algerian-like (type 0): 153 segregating (3 type 0:1 type 2): 69 like the Black Russian parent (type 2). The agreement to the expected 1:2:1 ratio attests to the accuracy with which the parental phenotypes could be distinguished (P = 0.95 − 0.50).

The cumulative evidence presented above indicates that the recombination value, if crossing over occurred, was less than 0.30 percent. Such a low value justifies considering the genes conditioning mildew resistance in Algerian and Black Russian as alleles since their expression can be distinguished phenotypically. In conformity with past procedures for assigning symbols to genes conferring resistance to powdery mildew, the allele in Black Russian has been designated Ml_{a2}.

Association between the gene conditioning resistance in Black Russian (Ml_{a2}) with those of Psaknon (Ml_p), Selection 175 (ml_d) and Kwan (Ml_k) was suggested in the test crosses (table 1). The recombination values between Ml_{a2} and Ml_p, and Ml_{a2} and ml_d, calculated from F_2 data (table 1) were 41.5 ± 2.7 and 17.5 ± 1.8 percent, respectively. The Ml_p and ml_d genes were reported by BRIGGS (1945) to be located in linkage group II with 16.4 ± 1.7 percent recombination. The suggested arrangement is as follows:

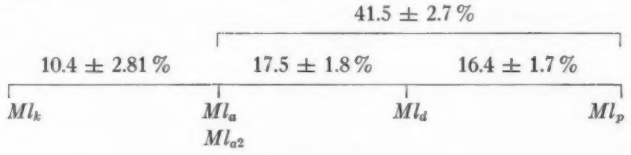


Although STANFORD and BRIGGS (1940) did not report association of the Ml_a locus with either Ml_p and ml_d, the present results are not in conflict with their data. Since the recombination value of 41.5 ± 2.7 percent between Ml_{a2} and Ml_p does not seriously modify the ratio expected with independence, large F_2 populations are necessary to detect it. Only 398 F_2 plants were tested previously in contrast to 1328 plants in the present study.

In the original test cross between Ml_a and ml_d, Duplex was used as the tester variety (STANFORD and BRIGGS 1940). In addition to ml_d, Duplex possesses the genes Ml_p and Ml_h for resistance. Consequently, with four genes segregating an extremely large population would be needed to detect the small departure from the

ratio expected with independence. Substitution of the recombination value of 17.5 ± 1.8 percent between Ml_a and ml_d in the original data gave a satisfactory fit to their observed segregation.

In the test cross with Kwan (Ml_k) 64 F_3 progenies were tested in addition to the F_2 population. Progenies from the singly dominant heterozygotes and the coupling phase heterozygote (with close linkage) would segregate in a ratio of 3 resistant plants:1 susceptible. Six segregating rows were observed. Only progenies from the double recessive F_2 plants would be homozygous susceptible. None were found. The combined estimate of linkage by the method of maximum likelihood from the F_2 and F_3 data gave a recombination value of 10.4 ± 2.81 percent between the Ml_{a2} and Ml_k loci. This estimate is in close agreement with the value of 9.81 ± 4.78 percent reported by BRIGGS and STANFORD (1938) for the Ml_a and Ml_k genes. Cumulative evidence places the Ml_k locus beyond the Ml_a locus in respect to Ml_p and ml_d since no association was detected between them in previous test crosses. With any other arrangement, the proximity of the genes with each other should have been readily established. Thus the following arrangement of the five genes in linkage group II is indicated:



DISCUSSION

Resistance to powdery mildew within the *Hordeum vulgare* species is world-wide in occurrence (MAINS and MARTINI 1932). The differential reaction of many varieties to physiologic races of the pathogen (NEWTON and CHERWICK 1947) suggests that the genetic basis of resistance is extremely diverse. Likewise, the genetics of pathogenicity must be equally diverse. Varieties immune to certain physiologic races are completely susceptible to others.

In the 13 varieties studied thus far, 10 genes have been identified which condition resistance to race 3 of powdery mildew. Incomplete results (SCHALLER unpublished) involving 5 other resistant varieties indicate at least 3 additional genes are present. These varieties represent a fairly random sample of the world germ plasm, including types from China, India, North Africa, Russia and Germany. The number of varieties examined has not been large enough to determine the frequency or the distributional pattern of the genes from any one source.

The nature of resistance imparted by the various genes has not been determined. Phenotypically, however, the majority of them can be readily identified, either by the level of resistance imparted or by their degree of dominance. The Ml_a gene appears to exclude the pathogen (immune reaction) and is completely dominant. The Ml_{a2} genotype permits limited mycelial growth and sporulation and is incompletely dominant. Resistance of the Ml_k gene appears to be one of hypersensitivity to the pathogen, whereby the plant cells adjacent to the infection points are killed, resulting in starvation of the pathogen. Variation in the nature of resistance is also

suggested by the differential gene-race interaction. Although this work is just beginning, it is apparent that the 10 genes exhibit considerable selectivity in regards to the different physiologic races.

It is clearly evident that the factors for resistance are not located at random in the 7 linkage groups. Five of the 10 identified thus far are in linkage group II, two forming an allelic series. Accumulated evidence (SCHALLER unpublished) suggested that one of the others is also in this linkage group, possibly an extension of the allelic series at the Ml_a locus. Only one of the remaining four genes (Ml_h) has been definitely placed in another group, group IV.

The non-random distribution of the genes conditioning resistance to race 3 of powdery mildew is parallel to that found by FLOR (1947, 1954) with flax rust and by BRIGGS and associates (BAKER 1949; BRIGGS 1940; STANFORD 1941) with bunt of wheat. Twenty-two genes imparting resistance to rust in flax have been placed in 3 series, either allelic or closely linked. Four of the seven genes for resistance to race T-1 of *Tilletia caries* (DC.) Tul. in wheat are located in one linkage group. The significance of this distribution pattern of resistance genes within a species is not readily apparent. If each of the genes conditioned resistance to different races occurring within an area, it can be readily seen that a chromosome carrying all of the genes would have a high selective advantage. However, in the majority of the varieties, resistance is conditioned by a single gene pair. If the genes in question have a pleiotropic action and their supplementary effects have a selective value, either independent or complementary, their association in a linkage group would then have a selective advantage. Further investigations with other physiologic races of the same pathogens and with other diseases and hosts are necessary to establish whether the pattern, both as to numbers and arrangements of genes for resistance, suggested by these studies is the rule or the exception.

SUMMARY

A single gene conditioned the resistance of the barley variety, Black Russian, to race 3 of powdery mildew. Segregation occurred in all test crosses, except with the variety Algerian which possess the Ml_a gene for resistance. Sufficiently large F_2 and F_3 populations of the Black Russian $\times$ Algerian cross were grown to detect recombination values greater than 0.3 percent. The gene in Black Russian was considered to be allelic to the Ml_a gene in Algerian, since no crossing over occurred and the two alleles were readily distinguished phenotypically. The gene in Black Russian was designated Ml_{a2}.

Ten genes have now been identified which confer resistance to race 3 of powdery mildew. Evidence was presented to show that at least 5 of the 10 genes are in linkage group II. Their suggested linear arrangement in the linkage group was given.

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GENETICS OF THE MONOECIOUS CHARACTER IN SPINACH[1]

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WHILE spinach (*Spinacia oleracea* L.) is usually considered to be dioecious, there is a continuous range of monoecious types as regards the proportion of pistillate to staminate flowers per plant. Sex determination in dioecious strains of spinach is controlled by a mechanism that acts as if it were a single factor pair; the pistillate plant is homozygous (*XX*), and the staminate plant is heterozygous (*XY*) (JANICK and STEVENSON 1954a). The inheritance of the monoecious character has been genetically interpreted by HIRATA and YAMAMOTO (1931), NEGODI (1934), MIRYUTA (1937), LORZ (unpub.), SUGUIMOTO (1948), and BEMIS and WILSON (1953). SUGIMOTO (1948) suggested that a single gene independent of the *XY* factors controls monoecism, while BEMIS and WILSON (1953) assume that there are two closely linked factors in addition to the *XY* factors. A study of progeny segregation from selected crosses involving pistillate, staminate, and monoecious types was attempted to clarify the genetic mechanisms that bring about the monoecious complex in spinach.

MATERIALS AND METHODS

The seed for the major portion of this study was harvested in 1947 and represented the third generation of selection within the variety Nobel. All plants were classified for femaleness[2] by estimating the percentage of pistillate flowers per plant, employing a system suggested by LORZ (unpub.). Seven classes were used, namely, 100, 95, 75, 50, 25, 5, and 0 percent female. The percentage of maleness was the difference between the percentage of femaleness and 100. A plant designated as 100 percent female was completely pistillate, and a plant classified as 0 percent female was completely staminate. Plants classified as 95, 75, 50, 25, or 5 percent female were monoecious, differing only in the ratio of pistillate to staminate flowers.

Selfing and crossing were facilitated by isolating selected plants in the greenhouse.

RESULTS

A number of lines grown in February 1951 were found to exhibit distinct patterns of inheritance, as can be seen in table 1. The variety Old Dominion segregated only staminate and pistillate plants; while the variety, New Giant Leaf, segregated all three types, pistillate, staminate, and monoecious. Two lines, 73A0,0,7 and 73A5,1,3, derived from monoecious selections, were particularly interesting. Line 73A5,1,3

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[2] The terms maleness and femaleness, while not botanically precise, were found to be convenient in referring to the proportions of pistillate to staminate flowers in spinach and consequently have been used in this study.

TABLE 1

Sex ratios of four lines of spinach showing distinct patterns of inheritance

Lines	Sex types		
	Staminate	Pistillate	Monoecious
Old Dominion	25	33	0
New Giant Leaf	5	3	1
73A5,1,3	0	11	9
73A0,0,7	0	0	24

TABLE 2

Distribution of progenies of monoecious plants derived from 73A0,0,7 when selfed and crossed with pistillate plants

Monoecious plant selfed									× Pistillate (100 percent female)								
Parental phenotype	Frequency distribution of progenies in phenotypic classes expressed as percentage of pistillate flowers per plant							Total plants	Mean value in percent	Frequency distribution of progenies in phenotypic classes expressed as percentage of pistillate flowers per plant							Total plants	Mean value in percent
	100	95	75	50	25	5	0			100	95	75	50	25	5	0		
	percent									*percent*								
5				15.4	57.7	26.9		26	23.5	36.0	62.0	2.0					50	81.7
25			13.3	33.3	46.7	6.7		15	38.7	68.4	24.6	1.8	3.5	1.8			57	85.3
75			23.9	37.0	37.0	2.2		46	45.8	65.7	17.1	11.4	5.7				35	82.4

segregated into both monoecious and pistillate offspring, while 73A0,0,7 was found to be completely monoecious.

Some of the monoecious plants derived from 73A0,0,7 were subsequently selfed and in some cases used as the pollen parent in crosses with pistillate plants from the variety Long Standing Bloomsdale. The segregation of progenies from some of these selfed monoecious plants and crosses is presented in table 2. Selections derived from the line 73A0,0,7 were found to be true-breeding for the monoecious character. Significantly, the F_1 progenies from the pistillate × true-breeding monoecious crosses were also all monoecious. These progenies were, however, quite different from the monoecious ones obtained from selfing the monoecious pollen parents. They consisted of highly pistillate monoecious plants, the greater bulk of which were scored as 95 or 75 percent female. In contrast, the S_1 progenies of the selfed monoecious pollen parents consisted of highly staminate monoecious plants, the greater bulk of which were classified as 50, 25, or 5 percent female.

Some of the monoecious F_1 plants from one of the pistillate × true-breeding monoecious (5 percent female) crosses derived from 73A0,0,7, as well as some of the monoecious S_1 plants, were selfed. Seven selections from the subsequent F_2 populations were selfed to give F_3 lines. These results are presented in table 3.

The S_2 progenies derived from 73A0,0,7 consisted of highly staminate monoecious selections as did the S_1 progenies. The F_2 progenies segregated in a ratio of 3 monoecious to 1 pistillate. The monoecious segregates could be grouped into two classes. One class consisted of plants that were phenotypically indistinguishable from the

TABLE 3

Distribution of progenies from a monoecious selection derived from 73A0,0,7 when selfed and crossed with a pistillate plant

Generation	Parental phenotype	Frequency distribution of progenies in phenotypic classes expressed as percentage of pistillate flowers per plant							Total plants	Mean value in percent	Chi square for ratio	
		100	95	75	50	25	5	0			3:1†	1:2:1‡
		percent										
S_1 (pollen parent selfed)	5				15.4	57.7	26.9		26	23.4		
F_1 (pistillate × monoecious)	100 × 5		36.0	62.0	2.0				50	81.7		
S_2 (11 S_1 plants selfed)					17.3	56.1	26.5		98	24.0		
F_2	75(1)	19.6	44.3	13.4	16.5	3.1	3.1		97	80.9	1.24	2.84
	75(2)	27.5	27.5	14.5	18.8	11.6			69	76.9	.12	1.88
	75(3)	27.6	34.5	22.4	8.6	5.2	1.7		58	82.8	.09	2.80
	75(4)	16.5	30.8	28.6	16.5	3.3	4.4		91	76.4	3.29	4.25
	75(5)	27.0	32.0	15.0	23.0	3.0			100	80.9	.12	.38
	75(6)	27.5	25.0	22.5	20.0	2.5	2.5		40	78.9	.03	.15
	95(1)	26.4	32.1	15.1	17.0	5.7	3.8		53	78.3	.01	.16
	95(2)	23.1	28.2	17.9	23.1	2.6	5.1		39	75.8	.01	.70
	95(3)	20.1	24.0	28.0	22.0	2.0	4.0		50	75.5	.43	.72
Total (F_2)		23.4	32.2	19.3	18.3	4.4	2.5		597	78.8	.68	.82
F_2	5(1)				37.5	37.5	25.0		8	29.4		
	25(1)				17.6	29.4	52.9		34	18.8		
	25(2)	23.7	3.5	17.5	12.3	22.8	20.2		114	53.0	.01	60.93**
	50(1)	25.0	15.6	15.6	9.4	15.6	18.8		32	62.3	0.00	6.75*
	75(1)	22.0	8.0	24.0	16.0	18.0	12.0		50	60.7	.11	9.20*
	75(2)	30.2	3.2	19.0	11.1	17.5	19.0		63	58.3	.64	27.15**
	75(3)	25.0	9.4	9.4	21.9	15.6	18.0		32	56.7	0.00	18.75**

† 3 monoecious: 1 pistillate.

‡ 1 monoecious (50, 25 or 5 percent female): 2 monoecious (95 or 75 percent female): 1 pistillate (100 percent female).

* Significant at the .05 level.

** Significant at the .01 level.

F_1 plants (scored as 95 or 75 percent female) and the other class was phenotypically indistinguishable from the S_1 and S_2 segregates (scored as 50, 25, or 5 percent female). The two classes appeared in a ratio of 2 to 1, and the F_2 ratio of 3 monoecious to 1 pistillate could be broken down into a ratio of 1 monoecious (50, 25, or 5 percent female) to 2 monoecious (95 or 75 percent female) to 1 pistillate (100 percent female). It was hypothesized that the highly pistillate monoecious plants (scored as 95 or 75 percent female) in the F_2 segregation were heterozygous for some factor controlling monoecism, and would segregate into monoecious and pistillate plants when selfed, while the more highly staminate monoecious plants (scored as 50, 25 or 5 percent female) were homozygous for this factor and when selfed would breed true for the monoecious condition. Of the seven F_2 selections that were selfed, two selections (scored as 5 and 25 percent female) were found to be true-breeding for the monoecious

character and gave progenies restricted to the 50, 25, and 5 percent female classes. Five other selfed selections, scored as 25, 50, 75, 75, and 75 percent female, gave progeny that segregated into a ratio of 3 monoecious to 1 pistillate. The segregating F_3 lines, however, did not fit the ratio of 1 monoecious (50, 25, or 5 percent female) to 2 monoecious (95 or 75 percent female) to 1 pistillate (100 percent female). This lack of fit may be explained as an environmental effect, since the high greenhouse temperatures under which these plants were grown bring about a shift towards maleness (JANICK and STEVENSON in press).

These results may be explained on the assumption that a single incompletely dominant gene, *M*, is responsible for the monoecious character. The precise relationship between the *M* gene and the *XY* factors cannot be established from these data. The results obtained can be explained by assuming that the *M* gene is independent of the *XY* factors and that the monoecious plants under consideration are genetically *XX*, then:

XXMM = true-breeding monoecious

XXMm = monoecious, more highly pistillate than *XXMM* and segregating

XXmm = pistillate

The assumption that the gene *M* is allelic to the *XY* factor would give identical results. Using the symbol X^m as the gene responsible for the monoecious character, then:

X^mX^m = true-breeding monoecious

X^mX = monoecious (segregating)

$X\ X$ = pistillate

An analysis of true-breeding monoecious × staminate crosses was carried out in order to determine the relationship between the factor responsible for the monoecious character and the *XY* factor pair.

The difficulty of analyzing monoecious × staminate crosses was overcome by the use of a dominant marker gene for red stem in the pollen parent. Nine white-stemmed monoecious plants derived from 73A0,0,7 (from line derived from plant classified as 75 percent female, table 2) were crossed with a single red-stemmed staminate plant from the variety Long Standing Bloomsdale. At the same time, two other sister monoecious selections were selfed. The true-breeding, white-stemmed monoecious × red-stemmed staminate crosses segregated red-stemmed progenies in a ratio of 1 monoecious to 1 staminate; while the two selfed monoecious selections gave only monoecious progenies. The monoecious plants derived from the monoecious × staminate crosses were generally more highly pistillate than those monoecious plants obtained from the selfed monoecious plants as shown in table 4, indicating that the staminate plant used was recessive for the *M* gene, i.e., *XYmm* or *XY*.

These results are compatible with either of the two hypotheses concerning the nature of the *M* gene. If the *M* gene is independent of the *XY* factors, and assuming the staminate plant from Long Standing Bloomsdale to be homozygous recessive

TABLE 4

Analysis of true-breeding monoecious × staminate crosses (I). Distribution of progenies from monecious selections derived from 73A0,0,7, when selfed and crossed with a staminate plant

Generation	Parental phenotype	Frequency distribution of progenies in phenotypic classes expressed as percentage of pistillate flowers per plant							Total plants	Mean		Chi square for 1:1 ratio†
		100	95	75	50	25	5	0		Entire progeny	Monoecious progeny	
		percent										
Monoecious plants selfed	50			6.2	34.4	37.5	21.9		32	32.3	32.3	
	25			10.5	42.1	36.8	10.5		19	38.7	38.7	
F_1 (monoecious × staminate)	25 × 0		10.0	24.3	11.4	5.7		48.6	70	34.9	67.8	.02
	50 × 0		5.9	35.3	13.7			45.1	51	38.9	70.9	.32
	50 × 0			29.4	11.8			58.8	17	27.9	67.8	.24
	50 × 0		13.3	26.7	13.3			46.7	15	40.7	76.2	0.00
	50 × 0		3.1	12.5	18.8	9.4		56.2	32	24.1	55.0	.28
	50 × 0			40.4	7.7	1.9		50.0	52	34.6	69.2	0.00
	75 × 0		7.4	21.4	17.8			53.6	28	31.8	68.5	.04
	75 × 0			28.0	16.0			56.0	25	29.0	65.9	.16
	75 × 0		10.3	31.0	10.3	1.7		46.6	58	38.7	72.4	.16
Total (F_1)			6.3	28.7	12.6	2.6		50.0	348	34.2	68.5	0.00

† 1 monoecious:1 staminate.

for this gene (*XYmm*) and that *Y* is epistatic to *M*, the true-breeding monoecious × staminate cross should yield progenies in a ratio of 1 monoecious to 1 staminate, namely,

$$XXMM \times XYmm \rightarrow 1\ XXMm : 1\ XYMm$$

1 monoecious : 1 staminate

If the *M* gene is allelic to the *XY* factors and the staminate plant was genotypically *XY*, the same results would be expected:

$$X^mX^m \times XY \rightarrow 1\ X^mX : 1\ X^mY$$

1 monoecious : 1 staminate

Under either hypothesis, the true-breeding monoecious × staminate cross should yield monoecious plants that are heterozygous, which when selfed, should give progeny in a ratio of 3 monoecious to 1 pistillate. The staminate plants, however, from the true-breeding monoecious × staminate cross when crossed on pistillate plants would be expected to give one of two distinct types of segregation, depending on which of the above hypotheses is correct. If the *M* gene is independent of the *XY* factor pair, the staminate plants (*XYMm*) from the above cross, when crossed on pistillate plants, should give progenies which segregate 1 monoecious to 1 pistillate to 2 staminate, namely,

$$XXmm \times XYMm \rightarrow 1\ XXMm : 1\ XXmm : 1\ XYMm : 1\ XYmm$$

1 monoecious : 1 pistillate : 2 staminate

TABLE 5

Analysis of true-breeding monoecious × staminate crosses (II)

A. Distribution of progenies of pistillate × staminate plants where staminate plants are the progeny of true breeding monecious × staminate crosses (table 4, F_1).

B. Distribution of progeny of selfed monoecious plant derived from the true-breeding monoecious × staminate cross (table 4, F_1).

Parental phenotype	Frequency distribution of progenies in phenotypic classes expressed as percentage of pistillate flowers per plant							Total plants	Mean value of monoecious plants
	100	95	75	50	25	5	0		
	percent								
A. 100 × 0		3.9	16.3	15.7	11.8	2.8	49.4	178	53.2
100 × 0	.8	10.4	10.4	17.6	3.2	.8	56.8	125	51.5
100 × 0		5.6	14.4	14.4	13.3	4.4	52.2	98	65.1
95 × 0‡	.6	3.4	12.3	12.8	16.2	9.5	45.2	179	43.7
B. 75 selfed	28.4	11.0	13.8	12.8	19.3	13.8	.9	109	46.3

‡ Pistillate plant reclassified as 95 percent female after crossing.

If the *M* gene is allelic to the *XY* factors, however, the staminate plant (X^mY) from the above cross, when crossed on pistillate plants, would give progeny which segregate 1 monoecious to 1 staminate, namely,

$$XX \times X^mY \rightarrow 1\ X^mX : 1\ XY$$

1 monoecious : 1 staminate

In order to distinguish between these two hypotheses, four staminate plants from the progenies of the true-breeding monoecious × staminate crosses were crossed on pistillate plants from the variety Long Standing Bloomsdale, and one monoecious plant from this cross was selfed as shown in table 5. All of these four crosses gave progenies which segregated in a ratio of 1 monoecious to 1 staminate, indicating that the *M* gene is allelic to the *XY* factor pair. Although the monoecious progenies were extremely variable and contained highly staminate monoecious plants, this may have been due to an environmental shift toward maleness. The pistillate plant recorded in two of the progenies is probably due to misclassification. The progeny from the selfed monoecious selection segregated in a ratio of 3 monoecious to 1 pistillate as would be expected under either hypothesis.

If the *M* gene is allelic to the *XY* pair, the staminate segregates from the pistillate × F_1 staminate crosses should be genetically *XY*, and when crossed on pistillate plants, should give progenies segregating staminate and pistillate plants in a 1:1 ratio. To test this, six of the staminate progenies from the pistillate × F_1 staminate crosses were crossed on pistillate plants from the variety Long Standing Bloomsdale. However, one plant that was believed to be staminate was reclassified later as monoecious and scored as 50 percent female on the basis of the seed set. This is a further indication that the highly staminate monoecious plants in the pistillate × F_1 staminate cross represents an environmental shift toward maleness.

The five pistillate × staminate crosses segregated progenies in a ratio of 1 staminate to 1 pistillate as shown in table 6, although in progenies of two of the crosses some

TABLE 6

Analysis of true-breeding monoecious × staminate crosses (III)

A. Distribution of progenies of a monoecious selection when selfed and crossed with a pistillate plant.
B. Distribution of progenies of pistillate × staminate crosses.

(The monoecious and staminate plants are derived from the progenies of pistillate × staminate crosses (table 5, A.) where staminate plants are derived from the progenies of true-breeding monoecious × staminate crosses (table 4, F_1).)

Parental phenotype	Frequency distribution of phenotypic classes expressed as percentage of pistillate flowers per plant							Total plants
	100	95	75	50	25	5	0	
	percent							
A. 50 selfed	29.2	31.2	17.7	16.7	4.2		1.0	96
100 × 50	50.0	35.6	7.1	1.9			2.9	104
B. 100 × 0	54.3						45.7	46
100 × 0	37.4	8.7	3.5	.9			50.4	115
100 × 0	34.3	21.9	1.0				49.8	105
100 × 0	49.5						50.5	109
100 × 0	45.1						54.9	102

of the pistillate plants contained some perfect or staminate flowers and were classified as monoecious (95 and 75 percent female). The monoecious phenotype of some of the progenies from these two lines might have been due to the presence of modifying genes in the pistillate parent from Long Standing Bloomsdale. The progeny obtained from selfing the monoecious plant that was first classified as staminate segregated in a ratio of 3 monoecious to 1 pistillate and, when crossed on a pistillate plant, gave progeny which segregated in a ratio of 1 monoecious to 1 pistillate as expected.

DISCUSSION

Sex determination in spinach appears to be controlled by a "switch" mechanism that acts as if it were a single gene with three alleles, Y, X^m and X. X^m is incompletely dominant to X, because plants that are heterozygous, X^mX, although phenotypically monoecious, contain a higher proportion of pistillate flowers than X^mX^m. The allele Y is completely dominant to X^m and X, because X^mY and XY types are staminate.

In addition to this major factor conditioning monoecism, there are also apparently many modifying genes quantitatively inherited affecting the monoecious character, for it has been possible by selection to obtain true-breeding monoecious lines that have high and low values of femaleness, as measured by the proportion of pistillate to staminate flowers per plant. In three generations of inbreeding, it was found possible to select plants whose progeny mean values were as high as 70 to 80 percent female and as low as 5 percent female (JANICK 1954).

If we assume that these factors determining sex expression in spinach actually represent three alleles at a single locus, and that the monoecious condition in spinach is more primitive than the dioecious, as is generally believed in flowering plants (LEWIS 1942), it is possible to suggest methods of gene action of these alleles from

their dominance relationships. Their allelism suggests that they control a single physiological function. Crosses involving diploids and tetraploids have shown that a single dose of Y causes the plant to be staminate even in combination with three doses of X (JANICK and STEVENSON 1954b and in press). Thus the Y allele appears to be definitely male determining as found in the Melandrium studies (WARMKE and BLAKESLEE 1940) and in contrast to the Drosophila pattern (BRIDGES 1939). The X allele can be described as an amorphic gene, because its action represents a loss of a "Y" function. This would explain why only a single dose of Y is needed to produce the staminate condition regardless of the number of X alleles present. The X^m allele permitting functioning of male- and female-producing substances appears to function in a similar manner as the Y allele but not as efficiently. X^m acts as a hypomorphic gene in contrast to the Y allele. This would explain why an increase in the dose of X^m results in a shift toward maleness, i.e., X^mX^m types are more highly staminate than X^mX types, as well as why X^mY types are staminate. Apparently, plants of the genotype X^mX are physiologically unstable and may be readily shifted by environmental conditions. Modifying genes affecting the proportion of staminate to pistillate flowers may be presumed to control other reactions affecting the same physiological end product.

Some of the progeny segregations reported by BEMIS and WILSON (1953) indicate that there is a gene independent of the XY factors that results in the formation of pistillate flowers on plants containing a Y gene. While this gene has not been observed in the lines investigated in this study, the existence of such a factor appears perfectly reasonable. It has been shown that staminate plants, genetically XY, may produce hermaphroditic flowers (JANICK and STEVENSON 1954a).

While this model of gene action is only suggestive, it appears credible and conforms to the experimental data. It does not seem unreasonable to assume that the X and Y alleles arose from the X^m gene by mutation, namely,

$$X \leftarrow X^m \rightarrow Y$$

The selective advantage of either mutation in the evolutionary process might come about by assuring cross pollination with a subsequent increase in vigor and variability. Furthermore, the assumption that the sex determining mechanism in spinach consists of a single gene with a number of alleles rather than a complex of genes as suggested by STOREY (1953) for *Papaya carica* and WESTERGAARD (1948) for *Melandrium album* helps explain the lack of heteromorphic sex chromosomes in spinach, which presumably only arise via the selective advantage of a crossover suppressor system.

SUMMARY

The monoecious character in spinach appears to be controlled by one major gene, X^m, which was found to be allelic to the XY factor pair. X^m is incompletely dominant to X. The Y allele is dominant to X and X^m. Methods of gene action of these alleles were hypothesized from their dominance relationships.

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NUCLEAR DISTRIBUTION IN CONIDIA OF NEUROSPORA HETEROKARYONS[1]

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IN *Neurospora* heterokaryons with two genetically different kinds of nuclei, some of the macroconidia are heterokaryotic and some are homokaryotic for either of the nuclear types. The relative frequencies of these three classes of conidia can be found experimentally if suitable genetic markers are present in the nuclei. It is reasonable to suppose that these frequencies are related both to the number and distribution of nuclei in the conidia and to the frequencies of the two nuclear types in the culture. PROUT *et al.* (1953) have tested the assumption that the different nuclei are distributed randomly, according to their frequencies, into conidia of each given number class. Three different heterokaryons were used, the conidia from a number of cultures of each being plated on differential media. All were assumed to have the same frequency distribution of conidia with different numbers of nuclei per cell. Values for the proportions of nuclear types were found which, when transformed into frequencies of conidial types on the randomness assumption, would give the best fit to the observed conidial frequencies. Even when the best fit was obtained, the observed frequencies of homokaryotic cells were ordinarily in excess of the expected.

Similar experiments on another heterokaryon will be reported here. In these experiments the nuclear ratio and the average number of nuclei per conidium were changed by varying the medium. Deviations from the expected conidial frequencies were observed, always in the same direction as those found by PROUT *et al.* Whether such deviations preclude satisfactory estimation of the nuclear ratio is questionable. A simple approximation for the nuclear ratio from plating data will be given, based on assumptions differing in some ways from those of the randomness hypothesis and suggested by the nature of the observed departure from randomness.

MATERIALS AND METHODS

The nuclear components of the heterokaryon used were arginine (29997A) and methionine-amycelial (4894-422A), the latter a double mutant combining a methionine requirement and a rather drastic morphological modification. These components form a nutritionally nonexacting heterokaryon of normal morphology. The stock was maintained on Neurospora minimal medium and transferred to agar slants having various amounts of added sorbose. Conidia were taken from these cultures, suspended in water, strained through fine glass wool to remove mycelial fragments, appropriately diluted, and added to pour plates of sorbose agar. The plating medium was Neurospora minimal with 1.0 percent sorbose and 0.1 percent sucrose and was supplemented with 50 μg of dl-methionine per ml or 50 μg of l-arginine per ml or both, as indicated. Colonies were counted after 4 days at 33°C. Conidia were stained for nuclear counts, using the technique described by HUEBSCHMAN (1952).

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TABLE 1

Frequency distribution of conidia with different numbers of nuclei

Expt.	Sorbose in minimal medium (mg/cc)	Nuclei per cell							Total cells	Av. no. of nuclei/cell, n
		1	2	3	4	5	6	>6		
a	0.00	92	364	313	130	51	30	20	1000	2.88
b	0.0003	64	329	316	166	61	25	39	1000	3.16
c	0.009	99	381	322	131	47	13	7	1000	2.71
d	0.039	101	356	308	129	60	29	17	1000	2.87
e	0.16	199	448	223	78	28	13	11	1000	2.39
f	0.62	208	447	224	77	25	10	9	1000	2.34
g	2.5	224	389	212	103	44	13	15	1000	2.48

TABLE 2

Colony counts on different media. Each count is an average of four plates

Expt.	Minimal medium	Methionine		Arginine	Methionine + Arginine		Correction factor for methionine plates
		Normal	Amycelial		Normal	Amycelial	
a	336	268	103	423	368	99	1.25
b	213	177	79	331	256	50	1.20
c	234	193	183	314	254	208	1.21
d	122	84	210	138	98	195	1.45
e	92	67	193	110	103	149	1.37
f	71	63	143	101	64	136	1.13
g	98	53	175	119	76	210	1.84

RESULTS

On the basis of preliminary observations, it was suspected that the nuclear ratio and number of nuclei per conidium for this heterokaryon would both be changed by addition of sorbose to the medium. This proved correct and afforded a means of varying these parameters over a suitable range. Seven different cultures were studied. Table 1 shows the distributions of numbers of nuclei in conidia from these cultures.

Samples of the same conidia were plated on minimal, singly supplemented, and doubly supplemented media. Heterokaryotic conidia can initiate growth on any of the media, but those homokaryotic for methionine-amycelial or arginine can grow only where the respective supplements have been added. Since the methionine-amycelial homokaryons are morphologically distinct, they were scored separately where methionine was supplied. The results are shown in table 2. There is a deficit in the colony count wherever methionine is present in the medium. The number of normal colonies on methionine medium should equal the number on minimal, but it is less in every case. Similarly, there are fewer normal colonies on doubly supplemented medium than on arginine medium, where equal numbers are expected. This anomaly was manifest in the appearance of methionine plates; the colonies, both normal and amycelial, were variable in size, ranging to the limit of visibility. Further incubation did not change this picture, but it was found in subsequent experiments that the use of l-methionine (instead of dl) and reduction of the concentration to 25 μg per ml would obviate the effect. On minimal and arginine medium the colony size

TABLE 3

Observed and expected frequencies of conidial types, with estimates of $\hat{p}$ *by the iterative method, first approach*

Expt.	Conidial types						T (Total)	Estimated p or β	χ^2	Degrees of freedom	Signif. level
	Ta Obs.	Ta Expected	Tb Obs.	$T\beta$ Expected	Tr Obs.	Tp Expected					
a	129	129	87	81.1	336	341.9	552	0.555	0.531	1	0.47
b	95	61.3	118	81.6	213	283.1	426	0.468	52.12	2	<0.01
c	222	186.8	80	51.1	234	298.1	536	0.654	36.76	2	<0.01
d	305	298.9	16	9.2	122	134.9	443	0.886	6.38	2	0.02–0.05
e	265	265	18	14.2	92	95.8	375	0.858	1.17	1	0.27
f	161	149.7	30	18.8	71	93.5	262	0.775	12.94	2	<0.01
g	324		21		98		443	0.876	2.54	1	0.11

was uniform. It cannot be ascertained from the counts whether the methionine inhibition applies equally to methionine-amycelial homokaryons and other conidial types, but one would suppose this to be the case from the appearance of the plates. The assumption was made that methionine inhibition did not affect the ratio of normal to amycelial colonies. It appeared also that the severity of the effect was variable from plate to plate. The data were therefore corrected in each case by setting the count of normal colonies on methionine medium equal to the count on minimal, and computing a new value for the count of methionine-amycelial colonies according to the ratio of normal to amycelial colonies on the methionine plate. The numbers of arginine homokaryons were estimated by subtracting the counts on minimal from those on arginine medium. The corrected data are given in table 3 as the observed numbers of the three cell types.

According to the random distribution hypothesis, the proportion, p, of nuclei of the first type and the proportions $C_1, C_2, C_3, \ldots$, etc. of conidia, having 1, 2, 3, . . . , etc. nuclei, are related to the proportion, α, of homokaryotic conidia of the first type; β, of the second type, and ρ, of heterokaryotic conidia by the equations

$$\alpha = C_1 p + C_2 p^2 + C_3 p^3 + \ldots + C_n p^n, \tag{1}$$

$$\beta = C(1 - p) + C_2(1 - p)^2 + C_3(1 - p)^3 + \ldots + C_n(1 - p)^n, \tag{2}$$

and

$$\rho = 1 - (\alpha + \beta). \tag{3}$$

To test the hypothesis, by using a (the observed values of α), C_1, etc., p was first estimated from equation (1) by successive approximation. This estimate of p and the total number of conidia, T, were then used to compute expected values, β and ρ in equations (2) and (3). These expected values were compared with the observed values, b and r, by the usual χ^2 test with one degree of freedom. Because of the manner in which p was estimated and because some of the observed values were obtained by subtracting plate counts, the computed statistic does not have a χ^2 distribution, even asymptotically. However, it was simple to compute and served as a sort of preliminary test of significance. The χ^2 computed in this manner will always be greater than

TABLE 4

Observed and expected frequencies of conidial types, iterative method of Prout et al

Expt.	Conidial types						$\hat{p}$	χ^2	Signif. level
	Ta Obs.	Ta Expected	$T(b+r)$ Obs.	$T(\beta+\rho)$ Expected	Tr Obs.	$T\rho$ Expected			
a	129	128.5	423	419.7	336	339.8	0.557	0.070	0.79
b	95	90.5	331	299.4	213	254.3	0.578	10.264	0.0014
c	222	220.1	314	293.3	234	258.6	0.715	3.819	0.051
d	305	304.4	130	134.5	122	126.2	0.875	0.235	0.63
e	265	264.7	110	108.1	92	94.3	0.860	0.088	0.76
f	161	160.7	101	93.7	71	79.4	0.813	1.454	0.23
g	324	323.8	119	116.0	98	101.3	0.878	0.183	0.67

TABLE 5

Comparisons of p *estimated by different means*

Expt.	Iterative method first approach	Approximation eq. (4)	Iterative method of PROUT *et al.*
a	0.555	0.556	0.557
b	0.468	0.463	0.578
c	0.654	0.659	0.715
d	0.886	0.864	0.875
e	0.858	0.846	0.860
f	0.775	0.763	0.813
g	0.876	0.861	0.878

the minimum χ^2 so that if the hypothesis is supported by the preliminary test, a second computation is not necessary. If the significance level for the preliminary test was below 10 percent, p was re-estimated by the more laborious method of minimum χ^2, by use of all three observed values simultaneously. It is felt that the sample sizes were large enough to minimize the effect of the error introduced by subtracting plate counts; i.e., the values of χ^2 which were obtained may be taken as fairly representative of those which would have been found if it had been possible to observe the numbers of the three cell types directly. As shown in table 3, the deviation from randomness was significant in four out of the seven cases.

As a second approach, the method of PROUT *et al.* was applied. The methionine plate was corrected as previously described and the number on the arginine plate was used directly. The χ^2 computed from classes Ta, $T(b+r)$, and Tr was minimized with respect to p and T. These results, summarized in table 4, confirm the conclusion from table 3; namely, that heterokaryotic cells are less frequent than expected and homokaryotic cells more frequent. The estimates of p by the two procedures are not greatly different.

DISCUSSION

Whereas it is clear that the randomness hypothesis often fails to explain the data, it is by no means evident that estimates of p by methods based on random distribu-

tion are correspondingly erroneous. A deficit of heterokaryotic conidia, with roughly proportional increases in both classes of homokaryotic cells, could lead to enormous deviations from random nuclear distribution with negligible change in the minimum χ^2 estimate, $\hat{p}$. The relation of p to the plating results might be hopelessly obscured if the assortment of nuclei were preferential, according to genotype, but this does not appear to be the case for any of the material which has so far been studied. The experimental findings suggest that the distribution of genetically marked nuclei departs from random in only one respect, a tendency for like nuclei to occur together. This tendency is not unexpected in view of the circumstances of conidiation. Conidia are formed at the terminal branches of the aerial hyphae, and the daughter nuclei arising by mitosis in these locations have little opportunity to become mixed in a random nuclear pool. Consequently, the multinucleate conidia will tend preferentially to include isogenic nuclei. Moreover, as PROUT *et al.* have pointed out, experimentally isolated hyphal tips are sometimes found to be homokaryotic. Perhaps the culture, at the time of conidiation, should be regarded as partly heterokaryotic and partly a mosaic of homokaryotic regions. Such a model leads without further assumptions to the observed deviations from the random distribution.

This type of departure from randomness has an important consequence from the standpoint of the estimation of p; namely, that the average nuclear numbers among the different cell types become more alike. If the average nuclear number were the same in the two types of homokaryotic cells, then the ratio of the types in a rather large segment of the nuclear population would be known. When random distribution is the rule, the nuclear composition of conidia of each number class follows the binomial distribution with the proportions of homokaryotic cells represented by only the first and last terms. This regime would lead to marked differences in average nuclear number among the three cell types. In general, the heterokaryotic cells would have the highest number of nuclei, and the more frequent homokaryotic type would have a higher average number than the less frequent. However, insofar as like nuclei occur together in excess of chance, there will be an increased frequency of homokaryotic cells with higher nuclear numbers. This will tend to equalize the nuclear numbers in the three classes, particularly in the two homokaryotic classes. The small variance of the typical nuclear distribution works in favor of similarity of numbers of nuclei among the classes of cells, although the presence of uninucleate cells, which must be homokaryotic, will create some difference in nuclear number between homokaryotic and heterokaryotic cells.

In view of the trend toward equality of nuclear number in the two homokaryotic types, the following approximation for p is developed. First, it is recognized that each heterokaryotic cell has at least one nucleus of each type; thus two nuclei per heterokaryotic cell are of known type. Second, the ratio of types among nuclei in excess of those of known type is assumed for the sake of approximation to be the same as the ratio of the cell types among homokaryotic cells, i.e., a/b. Let us consider a cell population of size N. The size of the corresponding nuclear population is $N\bar{n}$, where $\bar{n}$ is the average number of nuclei per cell. Among the heterokaryotic cells there are at least Nr nuclei of each type. The remaining number of nuclei in the whole population is $N\bar{n} - 2Nr$, and if we assume that in this group the proportion of nuclei

of the first type is $a/1 - r$, then the total number of nuclei of the first type would be $Nr + [N(\bar{n} - 2r)a/1 - r]$. Thus,

$$p \approx \frac{r(1 - r) + a(n - 2r)}{\bar{n}(1 - r)}$$

and also

$$1 - p \approx \frac{r(1 - r) + b(\bar{n} - 2r)}{\bar{n}(1 - r)}.$$

This approximation has the interesting property of becoming more nearly correct in principle as the departure from random distribution increases. At the same time, roughly proportional increases in both homokaryotic cell types will have relatively small effects on the minimum χ^2 estimate, $\hat{p}$, in comparison to the effects on χ^2 itself. It is therefore to be expected that approximation (4) and the minimum χ^2 estimates should be in fair agreement even when χ^2 is large. Rather close agreement is observed as shown in table 5. It has been pointed out by PROUT *et al.* that the quantity $a + r/1 + r$, which represents the ratio of the number of colonies on the first supplement to the sum of those on the first and second supplements, will also approach p as r decreases. From the uniform direction of the deviations from random distribution, it can be inferred that the true value of p lies somewhere between this ratio and $\hat{p}$. From similar considerations it can also be inferred that approximation (4), with its assumption of equality of average nuclear number per cell in the different homokaryons, should ordinarily yield estimates deviating slightly from $\hat{p}$ and from the true p in the direction of 0.5, but not so far in this direction as $a + r/1 + r$. Therefore, the frequently excellent agreement of approximation (4) with results determined by the random distribution hypothesis, particularly by the first approach, lends strength to the notion that p can be quite satisfactorily estimated by either procedure. The simplicity of the approximation makes it preferable for ordinary purposes.

SUMMARY

The distribution of genetically marked nuclei in macroconidia formed by heterokaryotic cultures of Neurospora is frequently nonrandom. The departure from randomness takes the form of an increased tendency for like nuclei to occur together in the same cell. This type of nonrandom behavior is not a serious obstacle to the determination, with fair accuracy, of the relative frequency of a given nuclear type. A simple approximation is developed by which such frequencies can be estimated from plating data and nuclear counts.

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A POPULATION ANALYSIS OF HETEROZYGOTE FREQUENCIES IN DROSOPHILA[1]

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WITHIN recent years considerable emphasis has been placed on the phenomenon of heterosis. In 1950 a conference devoted to a discussion of that topic was held at Iowa State College, and the proceedings of the conference were published two years later under the title of "Heterosis" (GOWEN 1952). The reader is referred to the first three chapters of this book for an historical account of heterosis. More recently RENDEL (1953) and HAGBERG (1953) have contributed significantly to our understanding of the problems of heterosis. Much of the three aforementioned publications and other papers concerned with heterosis has been devoted to the two principal theories offered as explanations for the underlying mechanism of heterosis: dominance theory and overdominance theory. The latter postulates that "heterozygosis" *per se* results in increased vigor while the former maintains that the covering up of deleterious recessive genes by beneficial dominants is sufficient to explain the phenomenon. RICHEY (1946) asserts that "the accepted basis for hybrid vigor has changed from the theory of physiological stimulation to that of the interaction of dominant favorable genes." However, CROW (1948) maintains that, "the likely alternative is that increased vigor is due to certain gene loci where the heterozygote is superior to either homozygote. . ." These two antithetical statements represent the present-day state of the problem. It should be remembered, however, as both RENDEL and HAGBERG emphasize, that the two theories need not be mutually exclusive.

CROW (1952) has pointed out that a single overdominant locus has a tremendously greater effect on variance in population fitness than a single locus with dominance or intermediate heterozygote. He states, "if such loci are at all frequent, they must be important. The question is: how frequent are they?" It is toward an answer to this question that the present experiments were undertaken.

MUTANTS

Six autosomal recessive mutants of *Drosophila melanogaster* were employed in the tests.

a. eyeless[2] (*ey*[2]) a mutant of the fourth chromosome located at 0.2 (4-0.2). The eye is reduced to ½ to ¼ wild type area with relatively little variability.

b. clot (*cl*) 2-16.5. Eye color is dark maroon to sepia-like, with darkening increasing with increasing age.

c. vestigial (*vg*) 2-67.0. Wings are reduced to vestiges of basal region, showing veins, but lacking the marginal areas.

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d. thread (*th*) 3-43.2. Arista is thread-like, deprived of side branches.
e. hairy (*h*) 3-26.5. Extra hairs on scutellum, along veins, on pleurae, and on top of head.
f. black (*b*) 2-48.5. Black pigment on body and tarsi and along wing veins, darkening with age.

These particular mutants were selected for several reasons. They represent a spectrum of morphological deviation from extreme (vestigial and eyeless) to more subtle (thread and hairy). All of the autosomes are represented. The mutants are readily classifiable and do not overlap wild type, desirable conditions when dealing with large populations. They were all accorded a rank of 1 (BRIDGES and BREHME 1944) which refers to excellence of their classification, location and viability. It was deemed necessary to select mutants whose viability was good in order not to eliminate the homozygous recessive too rapidly from the population. With the possible exception of vestigial all these mutants fulfilled this prerequisite.

METHOD

The population analysis was carried out utilizing a procedure suggested to the author by DR. A. BUZZATI-TRAVERSO (1947). The adult population was introduced into bottle 1. On the fourth day all flies from bottle 1 were shaken into bottle 2. On the seventh day all flies from bottle 2 were shaken into bottle 3. On the tenth day all flies from bottle 3 were shaken into bottle 4. These flies from bottle 3 shaken into bottle 4 represent the surviving adult population. Also shaken into bottle 4 were all flies which had hatched in bottle 1. These newly hatched flies represent the incipient F_1 generation. On the thirteenth day into bottle 5 were shaken: all flies from bottle 4 (adults and a few F_1), all flies hatched from bottle 1 (F_1) and all flies hatched from bottle 2 (F_1). This technique was continued every three days until the conclusion of the experiments. On the nineteenth day after emptying the contents of bottle 1 into the new bottle (7), bottle 1 was discarded. On the 22nd day bottle 2 was discarded, and so on.

Each mutant was separately analyzed employing the foregoing technique. The populations for each mutant were run in duplicate. A "high" line and "low" line were established in which the initial gene frequency of the recessive was 0.800 and 0.200, respectively. To inaugurate the high line 100 unmated adults were placed in bottle 1. These 100 flies were comprised of 80 recessive flies (40 females and 40 males) and 20 homozygous wild type flies (10 females and 10 males). The original adult population of the low line consisted of 20 recessive flies (10 females and 10 males) and 80 wild type flies (40 females and 40 males). The wild type stock used in these experiments was a non-isogenic Canton stock.

The bottles referred to in this test were ½ pint milk bottles to which was added the usual molasses-corn meal medium enriched with dry brewer's yeast. Tegosept-M (methyl-ester-parahydroxybenzoic acid) was also incorporated in the medium to keep down mold growth. Before the introduction of flies three drops of live yeast suspension were added to each bottle. All populations were maintained in an incubator at $25 \pm 1°C$.

At the beginning of every month each population was counted. The count consisted of all the flies in all the bottles of both the high and low lines. To estimate the

percentage of homozygous and heterozygous flies which constituted the phenotypically wild type class progeny tests were employed. One hundred wild type males were randomly selected from each line and individually mated to three recessive virgin females in one ounce glass vessels, "creamers". Four days later the parents were removed from the creamers and all surviving males were returned to the population. On about the twelfth day after the beginning of the progeny test the creamers were inspected for the presence or absence of recessive flies which indicated the heterozygosity or homozygosity of the tested male. All creamers in which no progeny resulted were classified as "sterile". This sterile class was the result of early death or sterility primarily of the male.

The creamers were kept in an incubator at 25 ± 2°C. However, several times the thermostat mechanism failed to function and the incubator became overheated. This resulted in the loss of much of the progeny test at various times and accounts for the unusually high number of "steriles" noted several times in the results.

ANALYSIS OF DATA

The results of the population counts of the six mutants are summarized in tables 1–6. It can be seen in a general way that the ratio of wild type flies to recessive flies increases with succeeding counts. Also, in the progeny tests the ratio of homozygotes to heterozygotes increases with succeeding counts. It should be noted that the difference between 100 and the sum of the homozygotes and heterozygotes of the progeny test represents the steriles. A more quantitative measure of the trend is

TABLE 1

Summary of eyeless experiment: Frequencies of eyeless and wild type flies and results of progeny tests. Frequency of recessive allele (q) *and test of goodness of fit to Hardy-Weinberg distribution* (χ^2)

Count	High line						Low line					
	Population count		Progeny test		q	χ^2	Population count		Progeny test		q	χ^2
	+	*ey*²	+/+	+/*ey*²			+	*ey*²	+/+	+/*ey*²		
1	1910	619	15	73	0.499	10.9***	1619	272	31	62	0.384	4.9*
2	1142	268	24	72	0.444	7.8**	1881	221	33	54	0.330	5.7*
3	1324	258	37	47	0.403	0.1	1037	98	35	53	0.305	7.0**
4	1614	159	37	52	0.306	5.1*	2029	160	51	45	0.272	0.7
5	1616	180	45	46	0.318	0.2	1209	101	38	29	0.276	0.0
6	2074	140	49	43	0.255	1.5	1718	84	49	33	0.219	0.7
7	2001	139	48	42	0.258	1.3	1570	71	48	39	0.215	3.7
8	2014	108	37	25	0.227	0.3	2333	111	54	35	0.215	0.6
9	1776	49	54	27	0.168	0.9	1457	54	43	45	0.201	13.2***
10	1974	79	56	34	0.199	0.9	1933	69	54	12	0.179	5.0*
11	1891	59	57	36	0.180	3.2	1977	79	54	36	0.200	1.9
12	2157	92	57	36	0.205	1.0	2111	65	54	36	0.179	4.3*
13	1774	53	40	29	0.178	5.0*	2213	58	60	29	0.163	1.0
14	2221	75	58	32	0.184	0.9	1828	47	56	23	0.160	0.1

* Significant at 0.05 level.
** Significant at 0.01 level.
*** Significant at 0.001 level.

TABLE 2

Summary of clot experiment: Frequencies of clot and wild type flies and results of progeny tests. Frequency of recessive allele (q) *and test of goodness of fit to Hardy-Weinberg distribution* (χ^2)

Count	High line						Low line					
	Population count		Progeny test		q	χ^2	Population count		Progeny test		q	χ^2
	+	*cl*	+/+	+/*cl*			+	*cl*	+/+	+/*cl*		
1	814	1010	2	94	0.748	12.2***	1459	307	15	73	0.427	20.4***
2	1409	305	27	68	0.426	5.7*	2086	418	29	62	0.412	3.7
3	1336	226	24	56	0.387	6.9**	1661	325	26	62	0.409	5.7*
4	1685	305	32	63	0.396	3.7	1483	361	28	55	0.444	0.8
5	2103	421	33	51	0.409	0.2	1684	382	28	52	0.444	2.2
6	2095	410	37	57	0.405	0.3	2110	439	38	56	0.415	0.0
7	1649	327	36	55	0.408	0.2	1750	361	43	46	0.411	1.6
8	1952	373	36	59	0.402	0.9	1510	331	28	49	0.425	0.5
9	1726	317	45	47	0.392	0.5	2124	364	36	51	0.384	0.4
10	3260	504	44	49	0.366	0.0	3055	404	38	55	0.344	2.4
11	2496	398	39	54	0.372	0.6	2480	294	40	51	0.328	1.7
12	2336	309	56	39	0.329	4.8*	2559	327	43	48	0.337	0.2
13	1997	273	41	53	0.349	0.9	2663	301	48	46	0.319	0.0
14	2312	247	47	43	0.311	0.0	2637	211	45	48	0.275	2.8

* Significant at 0.05 level.
** Significant at 0.01 level.
*** Significant at 0.001 level.

desired, however, and the value which will be chosen to express the population fluctuations is q, the frequency of the recessive allele. It is also necessary to calculate the goodness of fit of the observed data to those which are expected assuming the three classes of flies (homozygous normal, heterozygotes, and homozygous recessive) are distributed in accordance with the Hardy-Weinberg law.

The count of each population yielded four data:

1) total number of wild type flies.
2) total number of recessive flies.
3) number of homozygous wild type flies in progeny test.
4) number of heterozygous wild type flies in progeny test.

The expected numbers of the four classes, assuming the Hardy-Weinberg relationship, may be expressed as follows:

a) $N\,(1 - q^2) - n$ — Total number of wild type flies minus the number of flies selected for the progeny test.

b) Nq^2 — Total number of recessive flies.

c) $n\,\dfrac{1-q}{1+q}$ — Number of homozygous wild type flies in progeny test.

d) $\dfrac{2\,nq}{1+q}$ — Number of heterozygous wild type flies in progeny test.

Where N = total flies counted, n = total flies selected for progeny test, and q = frequency of recessive allele.

It is evident that only a value of the statistic q is required to obtain an expected

TABLE 3

Summary of vestigial experiment: Frequencies of vestigial and wild type flies and results of progeny tests. Frequency of recessive allele (q) *and test of goodness of fit to Hardy-Weinberg distribution* (χ^2)

Count	High line						Low line					
	Population count		Progeny test		q	χ^2	Population count		Progeny test		q	χ^2
	+	*vg*	+/+	+/*vg*			+	*vg*	+/+	+/*vg*		
1	1552	141	25	67	0.304	27.1***	2281	53	58	21	0.151	0.0
2	1547	35	31	46	0.175	36.9***	2376	44	46	46	0.153	28.9***
3	2247	42	42	50	0.157	36.3***	1972	60	44	54	0.190	26.7***
4	1723	19	56	36	0.126	17.6***	1551	21	55	27	0.128	5.8*
5	1853	38	48	40	0.159	16.1***	1865	19	49	37	0.125	25.1***
6	1832	16	61	28	0.109	9.5**	2189	15	58	16	0.088	1.9
7	2689	8	65	19	0.068	9.0**	2756	15	75	20	0.081	3.3
8	2368	12	77	19	0.078	2.6	2041	9	67	18	0.077	4.1*
9	2776	7	78	19	0.066	6.8**	2330	7	78	15	0.063	2.2
10	2659	5	83	8	0.044	0.0	2476	5	76	16	0.057	5.3*
11	2405	1	62	8	0.034	3.8	2895	3	81	11	0.041	2.8
12	2631	1	87	6	0.025	0.7	2458	0	74	13	0.038	11.0***
13	2465	3	80	5	0.033	0.1	2811	0	82	13	0.035	10.7**

* Significant at 0.05 level.
** Significant at 0.01 level.
*** Significant at 0.001 level.

TABLE 4

Summary of thread experiment: Frequencies of thread and wild type flies and results of progeny tests. Frequency of recessive allele (q) *and test of goodness of fit to Hardy-Weinberg distribution* (χ^2)

Count	High line						Low line					
	Population count		Progeny test		q	χ^2	Population count		Progeny test		q	χ^2
	+	*th*	+/+	+/*th*			+	*th*	+/+	+/*th*		
1	1547	125	22	72	0.294	39.6***	1008	74	23	65	0.289	33.2***
2	1840	168	17	60	0.303	32.1***	1368	123	20	57	0.303	25.0***
3	1745	136	30	52	0.279	13.9***	2292	234	32	50	0.299	7.7**
4	2095	202	31	57	0.304	12.2***	1841	166	17	47	0.297	20.5***
5	2269	227	26	56	0.309	15.2***	2106	203	25	36	0.300	4.2*
6	2471	198	27	58	0.281	21.3***	2020	246	37	47	0.332	1.3
7	1807	112	32	41	0.249	8.5**	1923	147	29	42	0.273	7.9**
8	2437	154	40	45	0.249	6.3*	2794	226	36	45	0.277	5.0*
9	2225	102	30	32	0.216	7.4**	2580	181	37	42	0.260	4.8*
10	2601	103	47	37	0.200	4.6*	2512	149	44	45	0.242	5.3*
11	2329	84	47	29	0.190	1.5	2262	112	49	37	0.221	1.9
12	1952	53	57	28	0.166	0.9	2589	96	42	43	0.198	12.6***
13	2163	51	49	30	0.159	4.9*	2773	104	48	41	0.196	7.5**

* Significant at 0.05 level.
** Significant at 0.01 level.
*** Significant at 0.001 level.

TABLE 5

Summary of hairy experiment: Frequencies of hairy and wild type flies and results of progeny tests. Frequency of recessive allele (q) *and test of goodness of fit to Hardy-Weinberg distribution* (χ^2)

Count	High line						Low line					
	Population count		Progeny test		q	χ^2	Population count		Progeny test		q	χ^2
	+	*h*	+/+	+/*h*			+	*h*	+/+	+/*h*		
1	770	1228	5	81	0.786	3.2	1511	268	27	65	0.395	7.8*
2	1320	1026	10	77	0.663	4.2*	1454	660	10	73	0.563	10.7**
3	1385	723	16	64	0.587	1.5	1563	685	17	67	0.554	3.0
4	1620	831	20	67	0.583	0.5	1494	877	15	63	0.609	1.1
5	1881	821	5	9	0.551	0.3	1681	788	15	63	0.567	2.8
6	1295	430	22	70	0.503	3.6	1506	552	18	67	0.521	4.3*
7	1633	487	28	68	0.481	1.5	1946	698	28	62	0.514	0.0
8	1878	484	28	55	0.454	0.5	1740	481	28	61	0.467	0.9
9	2012	355	26	66	0.392	9.1**	1920	426	30	58	0.428	1.3
10	1942	336	38	51	0.385	0.1	1915	397	36	56	0.415	0.2
11	2267	250	36	53	0.319	4.6*	2311	391	39	52	0.381	0.1
12	2410	237	47	43	0.300	0.1	2242	333	30	57	0.363	5.4*

* Significant at 0.05 level.
** Significant at 0.01 level.
*** Significant at 0.001 level.

TABLE 6

Summary of black experiment: Frequencies of black and wild type flies and results of progeny tests. Frequency of recessive allele (q) *and test of goodness of fit to Hardy-Weinberg distribution* (χ^2)

Count	High line						Low line					
	Population count		Progeny test		q	χ^2	Population count		Progeny test		q	χ^2
	+	*b*	+/+	+/*b*			+	*b*	+/+	+/*b*		
1	360	1769	23	73	0.906	76.1***	1627	348	17	39	0.422	2.5
2	1263	255	26	63	0.416	5.6*	1374	173	29	61	0.344	10.5**
3	1828	257	34	52	0.354	2.4	1570	201	31	56	0.343	6.4*
4	1358	119	29	58	0.298	16.3***	1831	183	33	54	0.308	8.2**
5	1325	196	28	37	0.361	0.4	1638	205	27	41	0.337	2.8
6	2312	362	34	60	0.371	3.7	2159	339	40	49	0.369	0.1
7	1780	245	33	50	0.351	2.4	2080	278	40	51	0.345	0.9
8	2082	297	39	55	0.345	2.7	2080	193	42	48	0.295	2.3
9	1934	221	24	53	0.327	12.2***	2584	250	30	58	0.304	13.7***
10	2313	286	42	49	0.333	0.6	2189	246	42	51	0.320	1.6
11	2444	262	44	51	0.313	1.4	2555	255	51	40	0.300	0.2

* Significant at 0.05 level.
** Significant at 0.01 level.
*** Significant at 0.001 level.

value for each of the four classes. These expected values may then be compared to the observed values by means of a chi-square test to determine the goodness of fit.

The value q was estimated by means of the Method of Maximum Likelihood. By this method we choose a statistic so that the estimated population should be that

for which the likelihood is greatest. FISHER (1946) points out that the chi-square which can be calculated utilizing the statistic estimated by the method of maximum likelihood will be almost identical to that chi-square calculated by the method of Minimum Chi-square. He further shows that the test of the goodness of fit is only valid when efficient statistics are used in fitting a hypothesis to the data, concluding that the method of maximum likelihood yields such an efficient statistic. It should be mentioned that in the calculations of goodness of fit there is only one degree of freedom involved. The four classes of data represent four degrees of freedom but one degree is lost in obtaining N, the total number of flies; one degree is lost in obtaining n, the total number of flies in the progeny test; and one degree is lost in estimating q, the frequency of the recessive gene.

Tables 1–6 include, also, the results of the calculation of q and the chi-squares computed using this statistic. The interpretation of the results will be discussed separately for each mutant with a general summary of the individual interpretations following.

INTERPRETATION OF RESULTS

Eyeless[2]. The immediate striking fact about the results of the eyeless experiment is the increase in the gene frequency of eyeless in the low line from an original frequency of 0.200 to a frequency of 0.384 (table 1). The best interpretation for this result would seem to follow from the assumption that the majority of first generation flies which comprise the population of flies at the first count are heterozygous for all the chromosomes. If this assumption were correct, then the progeny test would yield more flies heterozygous for eyeless than would be expected if the first generation population were randomly constituted. To justify the assumption that there would be more heterozygous flies than homozygous flies, we need only recall the composition of the adult population. It consisted of males and females homozygous for eyeless and males and females homozygous for wild type. Assuming random mating two classes of zygotes would be formed: homozygotes and heterozygotes, and if the mating is truly random, no excess of heterozygous zygotes should be produced. If we suppose, however, that the heterozygous zygotes have a higher adaptive value than the homozygous ones, there would result more heterozygous flies than expected in the first generation. This postulated higher adaptive value could result from a heterotic effect manifested in all the chromosomes due to the covering up of recessive genes lowering viability by dominants introduced by the other parent. It might be argued that these assumptions would lead to the result that the high line should also exhibit an increase in q in the first generation but this would not necessarily be the case. Assuming that the two homozygotes are selected against with the homozygous recessive being the most strongly selected against, it is easy to show that in the case of the high line which possesses an original $q = 0.800$, superiority of the heterozygotes would still result in lowering the frequency of q. Although there are no data to substantiate the assumptions in the foregoing arguments, nevertheless, the assumptions are not extravagant.

While the assumption has been made that mating is random this is not necessarily the case. There could exist negative preferential mating in which flies tend to mate

with flies of unlike genotype. This would, of course, increase the number of heterozygous zygotes formed by the adult population. On the other hand, there might exist positive preferential mating in which flies tend to mate with flies of similar genotype. A situation as this would decrease the number of heterozygous zygotes produced by the adult population. MERRELL (1949), summarizing the results of previous investigators, points out that in many instances mating is preferential and may be either positive or negative. In his experiments with sex-linked mutants of Drosophila MERRELL obtains results which also indicate non-random mating.

In the experiments reported here there are no data pertaining to mating behavior and thus, for simplicity, mating has been assumed to be random. However, this in no way eliminates the possibility that mating is preferential and, as will be mentioned later, this latter hypothesis is suggested by results with some of the other mutants.

It is noted (table 1) that the chi-square values obtained in the early counts are significant. In the high line no significant chi-square values were obtained after count 4, with one exception (count 13). The hypothesis assumed in the calculation of the chi-square value was that the population distributed itself in the proportion $p^2 + 2pq + q^2$. The poorness of fit, therefore, suggests that this hypothesis is not a valid one and, in fact, all significant chi-square values which were obtained resulted from an excess of heterozygotes.

Three hypotheses, aside from the hypothesis of non-random mating, could account for this excess of heterozygotes:

a) single-locus heterosis of $ey^2/+$.

b) genes closely linked to ey^2 which effect increased viability.

c) a preponderance of flies heterozygous for all chromosomes (thus also $ey^2/+$) due to the masking of deleterious recessive genes by dominants introduced by the other parent.

If the first hypothesis were correct, we would expect the superiority of the heterozygotes to be maintained throughout the experiment, there being no reason to assume a diminishing of a true unilocus heterotic effect. The results indicate that this is not the case. The second hypothesis, on the other hand, could lead to the results observed if we imagine that the linkage is progressively broken up as the experiment proceeds. However, since eyeless lies on the fourth chromosome whose total map length is considerably less than 0.2 units (STURTEVANT 1951), it would be necessary to assume tight linkage of any viability genes. It is considered unlikely that such close linkage would be so rapidly broken up and for this reason the second hypothesis is also rejected. The third hypothesis appears to be the most probable. It was pointed out how such a preponderance of heterozygotes might readily occur in the early generations. These heterotic chromosomal complexes should soon be randomized as the population continues breeding.

One exception is noted in the high line (count 13) and three exceptions are noted in the low line (counts 9, 10, 12). The most likely explanation which can be offered for these exceptions would seem to be that the deviations observed are due to sampling errors. In all there were 154 calculations of chi-squares and we would expect, by chance, that some of them would exceed the 0.05 level of probability and, perhaps, even the 0.01 level of probability. There were seven calculations considered excep-

tions which exceeded the 0.05 level of probability and one exception which exceeded the 0.01 level of probability. Also, three exceptions exceeded the 0.001 level of probability. It is considered unlikely that these last three represent only sampling errors and thus no explanation is offered for these three exceptions. It might be significant to mention that two of the three exceptions were observed in the high and low line of the same experiment of one month's count.

Clot. The results of the clot experiment (table 2) again show the phenomenon of the gene frequency, q, increasing from 0.200 to 0.427 in the first generation. The explanation proffered for the eyeless mutant would also seem to apply here. The actual data indicate that the progeny test contains a disproportionately high frequency of heterozygotes at the first count. The progeny test in the high line yielded 94 $+/cl$ to 2 $+/+$ while in the low line the ratio was 73 $+/cl$: 15 $+/+$. Superior adaptive value of flies heterozygous for all chromosomes is again suggested as the best hypothesis. Assortative mating of the adult population is by no means eliminated as an alternative explanation, for it may be that flies unlike in phenotype show more preference for mating with each other than flies similar in appearance.

It is observed, with one exception (high line, count 12), that no significant chi-square values were obtained after count 3, in either line indicating that the superiority of the heterozygotes is no longer manifested. The phenomenon responsible for the original superiority of the heterozygotes has apparently become randomized among all the flies and the population subsequent to the first few generations represents a randomly breeding population. This would be expected if the original excess of heterozygotes was due merely to multilocus heterosis.

Vestigial. The results obtained for the vestigial experiment are unique. It is the only mutant in which the gene frequency, q, has diminished rapidly, being at the end of the experiment 0.033 and 0.035 in the high and low lines, respectively, (table 3). This is clear indication that selection against flies phenotypically vestigial is severe. Also occurring in the vestigial results, different from all other mutants, is the lack of an increase in the gene frequency of vestigial in the low line from 0.200 to a higher value. This could arise from the fact that in this particular population there was no multilocus heterosis manifested in the first generation. Assuming this would be contrary to assumptions made for eyeless and clot. Notwithstanding, it remains a possible explanation. Another suggested explanation for the absence of an increase in q in the first generation might be related to the mating behavior of the adult population. It would be plausible to suppose that mating is not random when one class of flies possesses a morphological deviation from normal as extreme as vestigial. It is reasonable to believe that the 20 percent vestigial adults, or at least the males among these, were relatively unsuccessful in mating with the wild type flies. Superimposed on this postulated selective force would be the extreme selection against vg/vg zygotes which results from the mating of the vestigial adults among themselves. The net result of these two selective forces would be a decrease in the frequency of the vg gene in the next generation. The results of MERRELL's (1949) experiments with random mating in Drosophila would suggest that the postulated lack of mating of the vestigial flies might actually be the case. In his experiments males hemizygous for a sex-linked recessive gene were less successful in inseminating normal females than were their wild type brothers.

With respect to the goodness of fit to the Hardy-Weinberg distribution we observe in the high line that the significant chi-square values obtained persisted longer than for the eyeless and clot experiments and for almost the entire course of the experiment. These deviations were in every case due to an excess of heterozygotes. These results might be interpreted as the result of single gene heterosis. As was mentioned previously two phenomena which could lead to a persistence of excess heterozygotes would be single locus heterosis or closely linked genes which result in increased viability. Obviously, if the viability-increasing genes were very closely linked to the locus under investigation, it would become extremely difficult to differentiate between these two hypotheses. If, on the other hand, the linked genes were far enough away from the locus in question so that some recombination could occur over a long period of time we might expect that the excess of heterozygotes would disappear. In the high line of vestigial it would appear that the original excess of heterozygotes was, in fact, due to closely linked genes maintaining a preponderance of flies heterozygous for vestigial. After nine months (approximately 18 generations), however, recombination has been effective in breaking up these linkages and no longer is this excess of heterozygotes present. If the hypothesis of linkage is a correct one, chi-square values should not be observed for the duration of the experiment as is the case for the high line of vestigial.

The low line of the vestigial experiment presents results which are more disconcerting. In the first place, we note that the first count yielded results which conform almost perfectly with expectation. The next four counts and their concomitant chi-squares were similar to those obtained in the high line. Why the first count should agree so well with expectation while subsequent counts do not, is not readily explainable, unless the breeding structure of the adult population was in some way involved as previously suggested.

If we ignore the last two counts of the low line, it might seem that recombination has acted similarly to that postulated for the high line and that no significant excess of heterozygotes persists after the early counts. However, the last two counts (12 and 13) again yielded a significant excess of heterozygotes. The explanation for this is not readily apparent but it is conceivable that recombination would progress at different rates in the two lines and continuation of the low line experiment might have eventually resulted in no excess of heterozygotes. It should be mentioned in connection with the vestigial experiments that HERSH and WARD (1932) found that females heterozygous for vestigial have a significantly longer and larger wing than do homozygous normal females at 30°C. At 25°C the effect is the same although not significantly so. That this increase in wing size may be adaptive is speculative. The authors believed that the heterotic effect was due to the vestigial locus. However, an effect of genes at other loci cannot be excluded.

Thread. Of all the mutants tested thread alone yielded results which at first sight might indicate overdominance. An inspection of the data (table 4) reveals that for both lines all the chi-square values, except two in each line were significant. The exceptions may be merely chance deviations. In every case the deviation from expected was due to an excess of heterozygotes. These results could be readily interpreted as an example of single locus heterosis. However, the alternative of closely linked genes raising viability cannot be eliminated and careful analysis of the data

suggests that this is the more likely hypothesis. If we were dealing with true heterozygote superiority at one locus we would expect the population eventually to reach an equilibrium maintaining a rather constant value of q. The results indicate that this is not the case, at least as far as the experiment has progressed. The gene frequency of thread continually decreased. It is, of course, possible that the equilibrium point has not yet been reached. Inspection of the progeny tests shows us that the ratio of homozygous normal flies to heterozygotes is constantly decreasing, such that at the last count there were more $+/+$ flies than $+/th$ flies in both the high (49:30) and low (48:41) lines. Finally, it can be seen in the high line that the degree of deviation is decreasing as the experiment progresses. That is, the chi-square values are getting smaller with successive counts. (This is not true, however, in the low line.) All these facts are suggestive of the hypothesis of linked genes close to the thread locus which are raising viability. It would appear that as the experiment progresses recombination is yielding more and more flies without these linked genes, such that we might predict that in a few more generations the linkage would be completely broken up and the excess of heterozygotes would no longer be present.

Brief mention may be made of the fact that the frequency of the thread gene in the low line increased from 0.200–0.289. Multilocus heterosis is suggested as the reason for this increase.

Hairy. The results of the hairy experiments (table 5) give no indication of single locus heterosis. Of the seven significant chi-square values obtained three occur in the early counts and could be explained by multilocus heterosis as mentioned previously. The other four significant deviations, counts 9 and 11, in high line and counts 6 and 12, in low line might be ascribed to chance deviations.

Black. The results of the black experiments (table 6) are very similar to those obtained for eyeless and clot. Except for two unexplained deviations (count 9, high and low line) no significant deviations are obtained in either line after count 4, and thus there appears no evidence for single gene heterosis. Two interesting discrepancies, however, are observed in the results of the first count in both lines. In the low line we again observe an increase of q (0.200 to 0.422) but at the same time we have no significant excess of heterozygotes. In the high line the value of q also increased from 0.800 to 0.906 and the deviation from expectation is highly significant. In this one case, however, (the only such case in all the experiments) the deviation is due to a deficiency of heterozygotes. It is difficult to reconcile these facts without resorting to some special schemes of mating in the adult population. Since there are no data to suggest what these mating preferences might involve, it can merely be postulated that they may exist—by no means a satisfactory explanation.

Resumé. With the exception of thread none of the mutants maintained a significant excess of heterozygous individuals for the entire length of the experiment, although generally, an excess of heterozygotes was present in the early counts. This latter fact has been attributed to the phenomenon of multilocus heterosis. It is reasonable to assume that some inbreeding degeneration has occurred in a stock that has been maintained for hundreds of generations. In the event of such inbreeding degeneration in two different stocks considerable heterosis is to be expected in the hybrid individuals. All first generation homozygotes for the mutant gene or its normal allele

would be inbred like their parents so that their surviving offspring, like all the offspring in the F_1, might also be largely hybrids and hence more frequently heterozygous for the mutant than would otherwise be the case. Excess heterozygosis due to this cause might be expected to continue until that generation of flies whose parents were only rarely derived from a single one of the two parent cultures. In postulating this mechanism of multilocus heterosis resulting in excess of heterozygotes random mating has been assumed for the adult population. However, several inconsistencies have arisen in the results (vestigial experiment, low line, count 1; black experiment, high and low line, count 1) which might suggest that in some cases mating is assortative and not random. Unfortunately, no data are present bearing directly on this problem, although experiments by other authors offer evidence of non-random mating.

Should the hypothesis of multilocus heterosis be a correct one, we would not expect an excess of heterozygotes to persist, since the various genes raising viability should become randomized through the population by recombination. Only if these advantageous genes were closely linked to the locus under investigation might one expect a persistence of heterozygotes. The results indicate that an excess of flies heterozygous for the recessive mutant is not maintained with the exception of thread, and possibly the low line of the vestigial experiments. A close examination of the data of these two experiments suggests that here too we may be dealing with adaptive genes closely linked to the thread or vestigial locus, and that recombination is continually providing a more and more random distribution of these genes.

DISCUSSION

The results of the population experiments with the six autosomal mutants give no clear cut evidence for overdominance effects. It has been suggested in this paper that ordinary dominance considerations similar to those proposed by JONES (1917) are sufficient to explain the observed facts. It is, of course, valid to maintain that the six mutants selected for these experiments were, by chance, the "wrong" ones to select in an attempt to demonstrate overdominance. Only further experimentation with many more mutants could substantiate or remove this objection. The above argument concerning the wrong choice of mutants is perhaps strengthened by the evidence of MASING (1938, 1939a, 1939b) and TEISSIER (1942a, 1942b) revealing apparent single locus heterosis with the mutants they selected. It is believed by this author, however, that alternative explanations are possible and likely.

MASING tested lethals which he obtained from nature to determine if flies heterozygous for these lethals were more frequently found than would be expected. He did, in fact, find this phenomenon to occur and in one set of experiments 9 out of 19 tested lethals exhibited overdominance. MASING concluded that some lethal genes when heterozygous are capable of raising the viability.

The author would like to propose that the increase of flies which were heterozygous for the lethal gene was not due to the single locus itself but to many other loci in the genotype. In other words, the excess of heterozygotes was due to multilocus heterosis. This conclusion is suggested not only by the results of the experiments in this paper but by some further experiments and observations of MASING. The latter

points out that both the increased and decreased vitality of the heterozygotes diminishes with time, concluding therefore, that the analysis of the vitality of the heterozygotes for the lethals should be conducted immediately upon obtaining lethals from nature. This evidence of diminishing effect with time parallels well the data of the autosomal mutants here reported and would seem to be explained by recombination of these many loci responsible for raising the viability. In all the experiments so far mentioned MASING crossed the lethal gene to laboratory stock females. He also crossed the lethal bearing flies with females from nature, specifically from the same area in which the lethal gene itself was collected. In this latter experiment only two lines out of twenty-six showed an increased viability of the heterozygote. It may well be that the genetic background of the females collected from nature is more similar to that of the tested lethal bearing fly than would be the genetic background of an unrelated laboratory stock female. Were this the case we would expect a greater possibility of multilocus heterosis to occur with the laboratory female than with the female from nature.

TEISSIER (1942a) performed experiments similar to those of MASING. For his lethal gene he chose two genes in Drosophila, namely Curly (*Cy*) and Dichaete (*D*) which are dominant for marked morphological effects but which are lethal in the homozygous state. In none of the experiments with Dichaete was there any evidence for heterozygote superiority. With the Curly gene, however, several instances of overdominance were recorded. One resulted from the cross:

$$\text{P} \quad \frac{Cy}{+} \times \frac{+}{+}$$

$$\text{F}_1 \quad \frac{\text{Curly}}{8326} \qquad \frac{\text{non-Curly}}{7450}$$

It is seen that a significant excess of flies heterozygous for Curly is obtained. This, however, could very well result from multilocus heterosis of the sort already mentioned, particularly since *Cy* is associated with two inversions suppressing recombination. In two other experiments TEISSIER obtained the following results:

$$\text{P} \quad \frac{Cy}{vg} \times \frac{vg}{vg} \qquad \frac{Cy}{vg} \times \frac{Cy}{vg}$$

$$\text{F}_1 \quad \frac{\text{Curly}}{3424} \qquad \frac{\text{non-Curly}}{2712} \quad \frac{\text{Curly}}{4807} \qquad \frac{\text{non-Curly}}{1947}$$

It is here observed that the non-Curly flies are not only lacking the Curly gene but are, in fact, homozygous for vestigial. The results of the above experiments can thus be viewed from two aspects. Is there an excess of heterozygotes or a deficiency of flies homozygous for vestigial? The author's experiments with the vestigial mutant have shown that flies homozygous for vestigial are strongly selected against and it would appear that TEISSIER's experimental results involving *vg* and *Cy* might well be interpreted in this light. In other words, there is not an excess of flies heterozygous for *Cy*, but rather a deficiency of flies homozygous for vestigial. Concomitant with this phenomenon may be the effect of multilocus heterosis.

In a subsequent experiment TEISSIER (1942b) placed *Cy* into a population cage.

In one experiment the parents were *Cy/vg* × *vg/vg*, while in another experiment the parents were *Cy/+* × *+/+*. In the latter experiment the frequency of Curly flies diminished rapidly from 67 percent to 0.3 percent in 100 days. Certainly no evidence for heterozygote superiority is present. In the experiment involving vestigial the frequency of the Curly flies initially was 74.5 percent, decreasing only to 64.7 percent in 100 days. TEISSIER concludes that this is good evidence for superiority of the *Cy* heterozygote enabling the *Cy* gene to remain in high frequency. As mentioned previously, however, the chromosome bearing the *Cy* gene carries, also, two inversions suppressing crossing over and should there be genes linked to *Cy* which affect viability they could not readily recombine and thus the excess of heterozygotes cannot be easily attributed to the Curly locus itself. In conclusion it may be stated that the experiments of MASING and TEISSIER do not require the single locus heterosis hypothesis.

The results obtained by GUSTAFSSON and his co-workers (GUSTAFSSON 1946, 1947; GUSTAFSSON and NYBOM 1950; GUSTAFSSON, NYBOM and VON WETTSTEIN 1950) regarding overdominance in barley do not seem subject to the same criticisms as offered for the Drosophila experiments. GUSTAFSSON obtained spontaneous lethal mutants in highly inbred strains of barley and noted the superiority of plants heterozygous for these mutants when compared to their homozygous normal sister plants. The mechanism of multilocus heterosis cannot be easily invoked as an explanation for these results as the plants have had a long history of inbreeding and the genotypes of all plants are apparently very similar. The barley material would seem to present good evidence for overdominance.

BUZZATI-TRAVERSO (1952) reports that a gene for light eye in *Drosophila melanogaster* arose spontaneously several times in his stock bottles. Individuals homozygous for this same gene were found in nature. These facts suggested that the mutant had a selective value superior to that of the normal allele and experiments were undertaken to check this point. The results of these experiments indicated that the mutant for light eye was slightly superior to the normal allele and that natural selection has been acting in favor of the heterozygous flies. The experiments of TEISSIER (1947a, 1947b) revealing the persistence of the mutants ebony and sepia in population cages also suggests that selection is favoring heterozygotes. KALMUS (1945) likewise finds that ebony flies persisted in his populations over a long period of time and concludes that under the conditions of the experiment the heterozygotes were the fittest.

STERN, CARSON, KINST, NOVITSKY and UPHOFF (1952) in an extensive analysis of sex-linked lethals in Drosophila find that the majority of these lethals lower viability in the heterozygous condition. However, several mutants out of 77 tested apparently raise the viability when heterozygous and, thus, appear to manifest overdominance.

The evolutionary importance of overdominance in natural populations is significant. A higher adaptive value of the heterozygotes would maintain recessive genes in the population at a much higher frequency than if the heterozygote had an adaptive value lower than that of the homozygous normal individuals. Thus the population could contain sufficient genetic plasticity to allow exploitation of the numerous and diversified niches which it may encounter in its evolutionary lifetime.

The experiments in this paper were inaugurated to test for overdominance in

Drosophila. The data are best interpreted as lack of evidence for heterozygote superiority and further suggest that an excess of flies heterozygous for a recessive gene early in the experiment may be simply due to linkage phenomena. It is evident from the conflicting results of these experiments and those obtained by GUSTAFSSON and possibly TEISSIER, KALMUS, and BUZZATI-TRAVERSO that further analyses for overdominance must be undertaken.

SUMMARY

1. To test for overdominance, six autosomal recessive mutants of *Drosophila melanogaster* were separately analyzed utilizing a population bottle technique. These six mutants were: eyeless[2], clot, vestigial, thread, hairy and black.

2. Every month the entire population of each mutant was counted and 100 wild type males were randomly selected from each line for progeny tests to determine their genotype.

3. Employing the method of maximum likelihood an estimate of q, the frequency of the recessive gene, was obtained and the goodness of fit to a postulated Hardy-Weinberg distribution was determined.

4. All the mutants were characterized by an excess of heterozygotes during the first few generations. It has been postulated that this excess is due to multilocus heterosis, although non-random mating remains another possible explanation.

5. This excess of heterozygotes was not present in the later counts of the mutants with the exception of thread and possibly vestigial.

6. These latter two mutants presented, at first sight, evidence for overdominance but re-examination of the data suggests linkage of viability-raising genes as a more likely explanation.

7. The data which other investigators have advanced as evidence for overdominance are critically analyzed and hypotheses other than single locus heterosis are suggested for some of these data. However, other experiments in the literature seem best explained by overdominance.

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CHROMOSOME REJOINING CAPACITY WITH RESPECT TO BREAKAGE SENSITIVITY TO X-RAYS AND THERMAL NEUTRONS[1]

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THE degree of fragmentation caused by a given dose of X-irradiation on various stages of the nuclear cycle of *Trillium erectum* has been shown to vary by a factor of more than 50-fold between the least sensitive stage, early microspore interphase, and the most sensitive stages, diplotene and first meiotic metaphase (SPARROW 1951; SPARROW, MOSES, and STEELE 1952). One of the factors thought to provide a partial explanation for the observed difference in fragmentation was a differential ability of broken chromosome ends to reunite following irradiation at these various stages (SPARROW and MALDAWER 1950). In their study, stages of high and low sensitivity to breakage, namely, first meiotic metaphase and microspore interphase, were analyzed at microspore anaphase for three aberration types: acentric fragments which served as an index of the degree of sensitivity, and dicentric and ring chromosomes which measured the relative amount of rejoining. A high degree of reunion in the form of bridges and rings was found to occur following irradiation of cells at early interphase while a relatively low frequency of rejoins occurred at the more sensitive stage. However, this increased capacity for reunion of early interphase chromosomes was not sufficient to account for the observed 50-fold difference in total visible fragmentation between the two stages.

The present study was carried out to determine whether or not the apparent inverse relationship previously found to exist between breakage and rejoining would hold true at other stages in the nuclear cycle of Trillium. In addition, a comparison was made between X-rays and thermal neutrons in an attempt to establish the relative effectiveness of the two radiations on the induction of breakage and on the degree of rejoining.

MATERIALS AND METHODS

Cytological

Mature flowering plants of *Trillium erectum* L. were received in the fall of the year and were handled as previously reported by SPARROW and SPARROW (1949). At the desired stage of meiosis, determined by propiono-carmine smears of half anthers, anthers were irradiated either with X-rays or thermal neutrons. The stages irradiated were leptotene-zygotene, pachytene, diplotene, first meiotic metaphase, and early

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microspore interphase. As with the SPARROW and MALDAWER experiments, chromosome fragments were used as an index of breakage sensitivity and dicentrics and ring chromosomes were used to gauge the amount of reunion of broken ends. Cytological analyses of permanent propiono-carmine smears were made at first anaphase and at microspore anaphase to obtain data on the total amount of induced fragmentation and rejoining. An example of the type of microspore anaphase analyzed is shown in figure 1. This cell shown as a photograph and drawing was irradiated with 50r of X-rays at microspore interphase and contains two interlocked rings plus a dicentric and several fragments.

In order to understand the scoring procedure used, it is necessary to be familiar with the origin and fate of breaks and fragments during Trillium microsporogenesis. First, it should be clearly understood that *Trillium erectum* is somewhat unusual in that no typical resting nucleus is normally formed between first anaphase and second division. The chromosomes usually merely rearrange themselves at the end of first anaphase and then form the second metaphase plate. Thus, there is very little if any difference between the number of radiation induced fragments scored at first and second anaphase and it is certain that no increase in fragmentation occurs at the later stage. However, the situation with regard to bridges is not so simple since sister chromatid rejoining may lead to dicentrics which cannot appear as bridges until the second anaphase. But some first anaphase bridges not involving sister chromatid rejoins will have broken by second anaphase. These two complications make it difficult to get an accurate measure of the total number of bridges formed at meiosis. For this reason scoring at meiosis was done only at first anaphase.

Analysis of cells X-irradiated at prophase stages was made at both first anaphase and microspore anaphase while, as will be mentioned again later, prophase stages irradiated with thermal neutrons were examined only at first anaphase. It has been found in Trillium that just a partial picture of the whole irradiation effect can be observed at first anaphase when actual breaks appear immediately and that only after the chromosomes have passed through microspore interphase do the potential

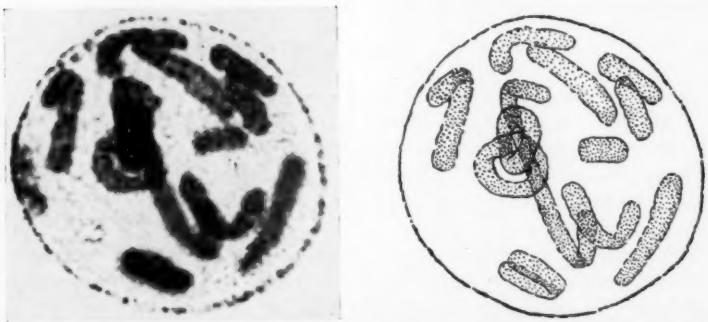

FIGURE 1.—Photograph and drawing of cell at microspore anaphase which demonstrates the presence of a dicentric, two interlocked rings, and several fragments. The cell received 50r of X-rays at microspore interphase.

POSSIBLE FRAGMENTATION RESPONSES IN TRILLIUM AFTER MEIOTIC PROPHASE IRRADIATION

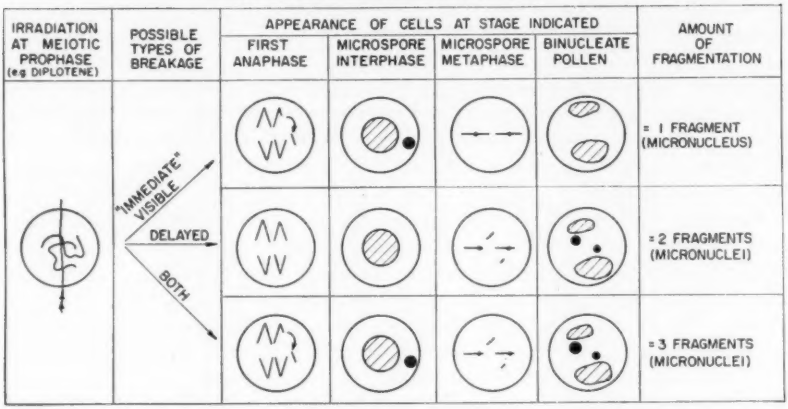

FIGURE 2.—A diagram indicating the possible appearance of cells irradiated at meiotic prophase (e.g. diplotene) in Trillium. Top row: the production of fragments immediately visible at first anaphase which form micronuclei at microspore interphase. Second row: formation of no immediately visible breaks but only potential breaks that become visible after the chromosomes pass through an interphase. Bottom row: combination of both immediate breaks at first anaphase and delayed breaks at microspore anaphase. It should be understood that the numbers of immediately visible and delayed breaks vary independently from cell to cell.

breaks become visible (fig. 2). Fragments that were first visible in meiosis usually appear as micronuclei during microspore interphase and eventually disintegrate in the cytoplasm. For the most part, fragments scored in the microspore divisions were not carried over from first anaphase of meiosis but were a new set of fragments which existed in meiosis only as potential breaks. Throughout the paper "fragments" include all types of acentric fragments (and/or deletions) and rejoins are the sum of rings and dicentrics regardless of origin.

Radiological

Some X-irradiations were done at the Biological Laboratories of Harvard University and the remainder at Brookhaven. Three different X-ray machines were used but in all cases the treatments were with 160 KVP X-rays at 10 or 15 ma. The half value layer varied from 0.12 to 0.3 mm Cu. The standard dose of 50r was delivered to the specimens in approximately 35 to 45 seconds at a distance of 50 cm from the target.

The thermal neutron irradiation was carried out in the thermal column of the Brookhaven nuclear reactor. Anthers at known stages of meiosis were irradiated for ten minutes with average neutron fluxes which ranged between 4.3 and 6.5 $\times$ 10^8 thermal neutrons per cm^2 per second. The cadmium ratio in the thermal column was about 5000:1 and the gamma contamination approximately 120r per hour. A standard lucite irradiation box, approximating an eight inch cube, was used to house a single

layer of the irradiated biological samples, and contained up to thirteen small vials when used in the experiments described below. The box was filled with nylon yarn to reduce temperature fluctuations during exposure. A gold foil was inserted with the vials to provide a measure of the neutron flux during the time of exposure; the amount of activation found in the foil was proportionally related to the dose applied.

Cultural

The X-ray data were obtained from buds irradiated on the plant but numerous technical difficulties were encountered when irradiating intact plants with thermal neutrons. For this reason, observations on thermal neutron irradiated cells were made from anthers removed from the buds and permitted to continue meiosis in sterile culture. Two media were tested for their ability to act as a suitable substrate; the primary criteria of fitness were viability or survival of the anthers and the frequency of spontaneous chromosome fragmentation.

Studies were made testing the effect of placing the anthers or the flower stalk on one percent agar and one percent agar plus White's medium (WHITE 1943). Results were compared with those obtained by leaving the plant intact, that is, allowing the anthers to remain in the bud and the bud on the rhizome. Anthers kept on the flower stalk in White's medium frequently became contaminated by bacteria or mold making this technique impractical. The anthers taken from the one percent agar medium had a white waxy color rather than a normal yellow appearance. Material grown directly on White's medium plus one percent agar resembled most closely development of anthers left intact on the rhizome.

The results of scoring at first anaphase and microspore interphase for spontaneous aberration frequencies in unirradiated Trillium anthers under various environmental conditions are presented in table 1. The data indicate that the environment of the anthers had little or no effect on the spontaneous aberration frequency. It was, therefore, decided that the culture technique was reliable and that a promising substrate for chromosome studies was one percent agar plus White's medium. Despite

TABLE 1

Effect of environment upon the frequency of spontaneous aberrations in Trillium anthers

Environment	Stages scored				
	First anaphase			Microspore interphase	
	Number of cells scored	Fragments per 100 pollen mother cells	Bridges per 100 pollen mother cells	Number of cells scored	Micronuclei per 100 pollen mother cells
In vivo (Intact plant)	1780	2.08	0.39	2000	4.60
In vitro					
a. Anthers on 1% agar	—	—	—	2000	4.20
b. Anthers on 1% agar + White's medium	1700	1.88	0.24	2000	3.80
c. Anthers on flower bud-flower stalk in 1% agar + White's medium	1000	1.80	0.10	1000	4.40*

* All other figures based on 10 plants; this figure is based on 4 plants.

the apparent suitability of the medium for chromosome studies, it was found that approximately thirty percent of the artificially cultured anthers were either partially or entirely sterile. Further experiments in this laboratory have been carried out and a more successful substrate for excised anthers has been found to be the medium used by TAYLOR (1950) supplemented with coconut milk (SPARROW, POND, and KOJAN 1955).

A further test of the culture system was made by a comparison of the number of fragments observed in anthers irradiated intact on the rhizome and the number of fragments observed in anthers excised from the bud prior to the irradiation and then grown in culture. There was no indication that a significant difference in breakage sensitivity existed at first anaphase as a result of the environmental differences.

Having established the practicality of the culture technique, a method was devised for the handling of large numbers of anthers. In preparation for irradiation,

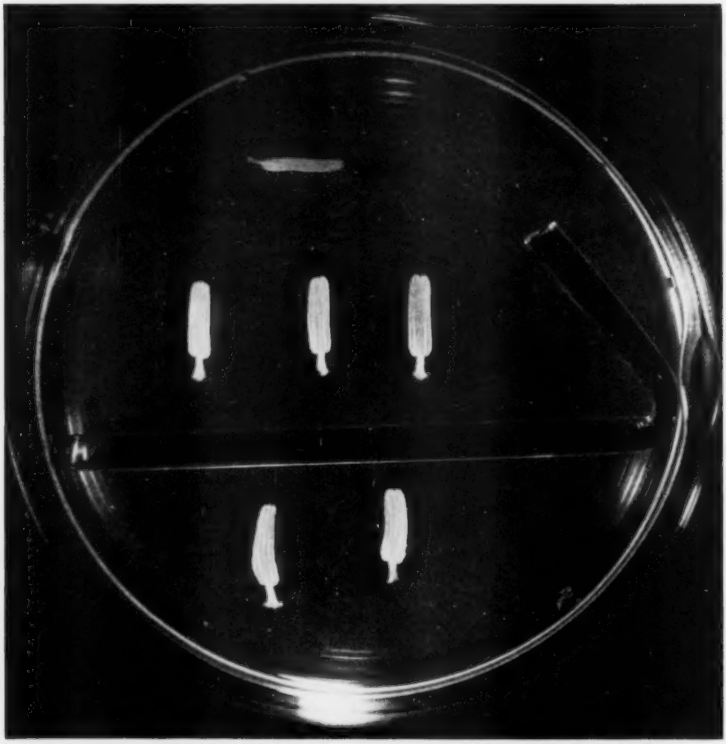

FIGURE 3.—Photograph of two inch Petri dish showing the relative position of anthers in relation to a glass rod used to separate different treatments involved. Three anthers on the hooked side of the rod have received thermal neutron treatment, two outside the hook have received X-irradiation, and the control lies parallel to the glass rod.

the flower buds were cut off the rhizomes, washed, and refrigerated at 4 to 6°C. They were removed from cold storage one at a time, introduced into a transfer chamber and dissected aseptically by first dipping the inflorescences into 70% alcohol and then removing the sepals and petals under ultraviolet light.

When meiosis had reached the desired stage, as determined by smearing a half anther, the five anthers were divided into two lots and placed in moist, sterile, polystyrene, shell vials approximately three-fifths of an inch wide and two inches long. Three anthers from each bud were exposed to neutrons in the thermal column of the nuclear reactor, and two were exposed to X-rays to determine possible changes in X-ray sensitivity as a result of the removal of the anthers from the rhizome. The unirradiated half anther was used as a control.

After irradiation the anthers were placed on one percent agar plus White's medium. In order to standardize procedure and to minimize environmental differences the anthers were arranged in a Petri dish in such a manner that the three anthers previously irradiated at the nuclear reactor were placed on the hooked side of a glass rod, the X-ray anthers outside it, and the control anther at right angles to the thermal neutron material (fig. 3).

RESULTS

Breakage and rejoining after exposure to X-rays

Data obtained from material irradiated with 50r of X-rays at leptotene-zygotene, pachytene, diplotene, first metaphase, and early interphase and scored at first anaphase and at microspore anaphase are presented in table 2. The results from first anaphase scoring showed that the average number of fragments per 100 cells found after irradiation of leptotene-zygotene, pachytene and diplotene (90, 120

TABLE 2

Comparative frequencies of fragments and rejoins induced by 50r X-rays during various stages of microsporogenesis in Trillium erectum

Stage irradiated	Number of cells scored	Stage analyzed†	Fragments		Rejoins‡		X-ray ratio	
			Number observed	Average number per 100 P.M.C.	Number observed	Average number per 100 P.M.C.	fragments / rejoins‡	
Leptotene-zygotene	2500	A_I	2259	90	828	33	2.7	
Pachytene	2700	A_I	3246	120	987	37	3.2	3.3
	1025	MSPA	95*	37	27*	11	3.4	
Diplotene	2700	A_I	3281	121	2240	83	1.5	3.8
	360	MSPA	381*	423	56*	62	6.8	
Metaphase I	400	A_I	16	4	2	0.5	8.0	8.6
	1275	MSPA	1915*	601	210*	66	9.1	
Early post-meiotic interphase	2700	MSPA	257*	37	69*	10	3.7	

* Multiply by four for direct comparison with first anaphase analysis, since each P.M.C. yields 4 microspores.

† A_I = first anaphase and MSPA = microspore anaphase.

‡ Rejoins include rings and dicentrics only.

and 121, respectively) were not significantly different. Cells irradiated at diplotene displayed much more fragmentation at microspore anaphase (423) than at first anaphase (121). This is the reverse of the trend found after pachytene irradiation where 120 fragments were found at first anaphase for every 37 found at microspore anaphase. After first metaphase irradiation the fragment production reached 601 per 100 pollen mother-cells when scored at microspore anaphase.[4] However, this increase when compared to the diplotene value (423) was not a statistically significant one. Following postmeiotic interphase irradiation the fragmentation had fallen to a low of 37 per 100 pollen mother-cells. This is the least sensitive stage as far as fragmentation is concerned.

The data on rejoins (rings + dicentrics) given in table 2 will be discussed separately as (1) the average number per 100 pollen mother-cells and (2) as the ratio of fragments to rejoins (F/R ratio). The averages show the highest value for rejoining occurs after diplotene irradiation, followed by first metaphase, pachytene, leptotene-zygotene and postmeiotic interphase. The diplotene values (83 and 62) are not significantly different from each other nor from the metaphase value (66) but all three are significantly higher than both earlier prophase and interphase values. Taken as a whole these data show that the average number of rejoins produced is not systematically or consistently related to the number of fragments observed since stages which yielded almost identical numbers of first anaphase fragments (pachytene 120, diplotene 121) yielded very different amounts of rejoining (pachytene 37, diplotene 83). Similarly the meiotic and microspore scorings following diplotene irradiation gave significantly different fragment counts (121 and 423) concomitant with numbers of rejoins which were not significantly different (83 and 62).

The data pertaining to the ratio of fragments to rejoins is presented in the right hand column of table 2. These values also illustrate the fact that the rejoining is not related directly to the amount of fragmentation in any consistent fashion. It is true that the highest ratios were found in the two stages showing the highest amount of fragmentation (microspore scoring following diplotene and metaphase irradiation) but the lowest ratios (2.7 and 1.5) were not found in the least sensitive interphase stage. It seems clear, therefore, that in our material F/R ratios also vary from stage to stage in an unpredictable manner. These results would seem to negate the existence of a *consistent* relationship between the amounts of fragmentation which persists to the stage of scoring and the amount of rejoining detectable as rings and dicentrics.

Fragmentation and rejoining after exposure to thermal neutrons

With the culture technique established as a feasible method for handling excised anthers following their exposure to thermal neutrons, anthers containing cells at

[4] Cells irradiated at first metaphase and analyzed at first anaphase revealed few fragments and bridges, e.g. of 400 cells scored only 16 fragments and 2 bridges were found. Since chromosome fragments occur spontaneously in an average of about 2 percent of first anaphase cells (SPARROW and SPARROW 1950), the actual number of induced aberrations produced is open to question. For this reason plants irradiated at first metaphase or later stages were examined only at the microspore division.

zygotene, pachytene, diplotene, first metaphase, and early interphase were irradiated with neutrons for ten minutes. As previously indicated, these cells were exposed to wide variations in neutron flux. Three sources of evidence indicate, however, that these variations do not invalidate the data. First, analyses of differences between

TABLE 3

Comparative frequencies of fragments and rejoins induced by ten minute thermal neutron exposures at various stages of microsporogenesis in Trillium erectum

Stage irradiated	Stage analyzed†	Number of cells scored	Fragments		Rejoins‡		Neutron ratio $\frac{\text{fragments}}{\text{rejoins‡}}$
			Number observed	Average number per 100 P.M.C.	Number observed	Average number per 100 P.M.C.	
Zygotene	A_I	1200	2128	177	766	64	2.78
Pachytene	A_I	1800	2784	155	805	45	3.46
Diplotene	A_I	2100	1172	56	800	38	1.49
Metaphase I	MSPA	600	322*	215	27*	18	11.92
Early interphase	MSPA	1000	236*	94	40*	16	5.9

* Multiply by four for direct comparison with first anaphase analysis, since each P.M.C. yields 4 microspores.

† A_I = first anaphase and MSPA = microspore anaphase.

‡ Rejoins include rings and dicentrics only.

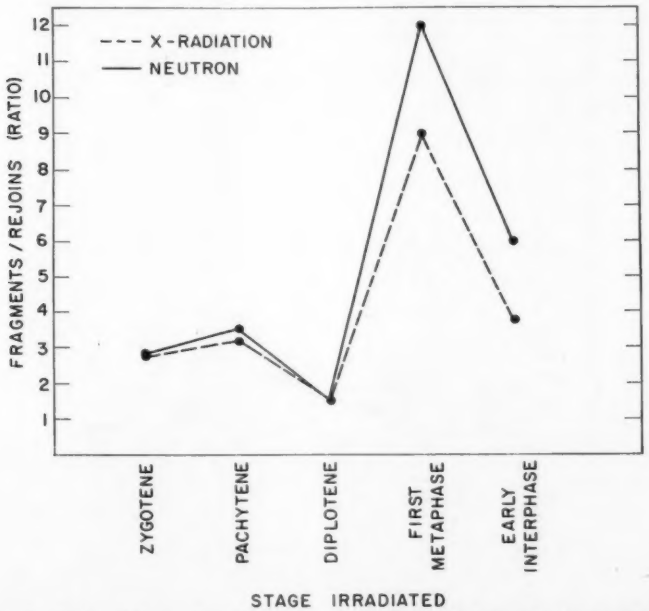

FIGURE 4.—Ratios of the number of fragments per rejoin obtained for each stage irradiated with X-rays and thermal neutrons.

fluxes and within each flux for each stage irradiated, showed no significant deviation from the expected in either the amount of fragmentation or in the number of rejoins. Secondly, analyses of covariance resulted in a small non-significant adjustment in both fragments and rejoins. The third possible source of error may be related to the method of determining the neutron flux by activation of gold foils. However, the counting technique was performed throughout by one person and the calculations carefully checked to minimize this source of error. In view of the above considerations, it seemed justifiable to disregard such flux variations in these experiments.

The amount of fragmentation and rejoining (rings and dicentrics) induced by ten minute exposure to thermal neutrons is summarized in table 3. The data indicated that first meiotic metaphase cells were more sensitive to thermal neutrons than zygotene, pachytene, or early interphase cells, but it should be pointed out

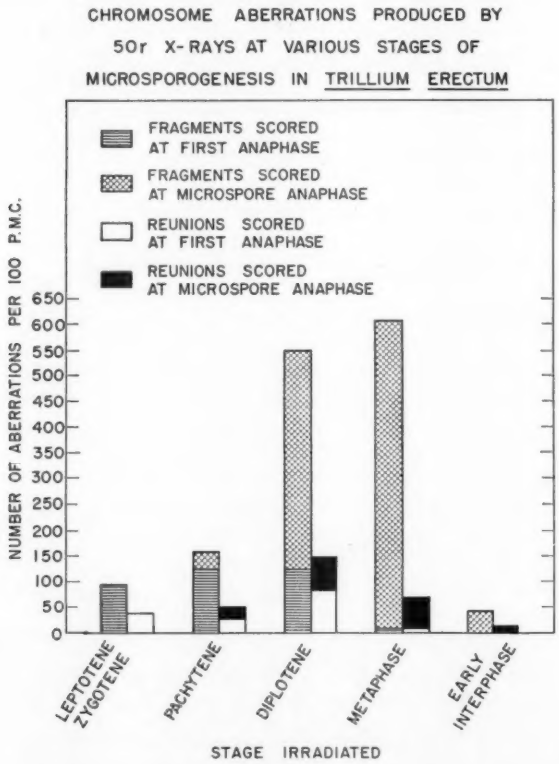

FIG. 5

FIGURES 5 AND 6.—Histograms indicating the number of fragments and reunions per 100 pollen mother cells obtained by irradiation of various stages with 50r of X-rays and ten minutes of thermal neutrons (4-7 × 10^8 thermal neutrons/cm^2/sec).

that microspore anaphase was not analyzed following diplotene irradiation and therefore total diplotene sensitivity based on induced fragmentation is not known.

The average number of rejoins per 100 pollen mother-cells that occurred at zygotene, pachytene, first metaphase, and early interphase were more or less constant for that stage regardless of the mean number of fragments induced, while at diplotene the number of rejoins appeared to be a function of the number of fragments produced. These generalizations are, however, based on a limited range of fragmentation, and speculation beyond this range would be hazardous.

Figure 4 shows the ratios between the number of fragments and the number of rejoins which were found at various stages after treatment with both X-rays and thermal neutrons. Following neutron irradiation at diplotene, one reunion occurred for every 1.5 fragments produced, while only one reunion per twelve fragments occurred after first metaphase irradiation. The striking similarity found between the ratios for materials treated with the two radiations appears worthy of emphasis.

Comparison of X-rays and thermal neutrons

On the basis of chromosomal aberrations reported above, thermal neutron irradiated material exhibits a more uniform response than X-irradiated cells, which

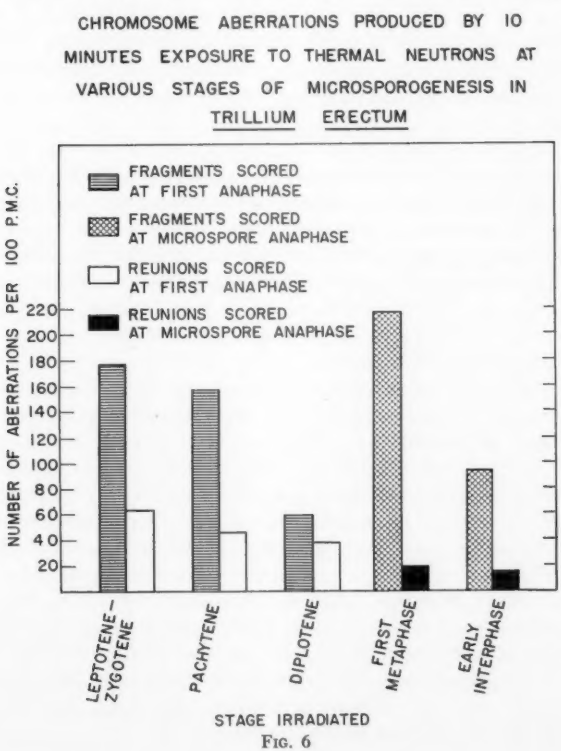

FIG. 6

vary by a factor of more than twenty-fold between the most sensitive and the most resistant stage, interphase (fig. 5). The comparable factor for thermal neutrons was only four-fold (fig. 6). The twenty-fold difference with X-rays is not to be construed as a contradiction of the sixty-fold difference in sensitivity between diplotene and early interphase previously reported (SPARROW 1951); his classification of various phases of interphase was more precise and based on a carefully followed time sequence in which cells irradiated at seven day interphase were found to be less sensitive than later stages of interphase.

The number of rejoins differs by a factor of eight between the extremes in the X-irradiated material, while the differences were less noticeable in the material treated with neutrons, a factor of four existing from stage to stage (fig. 6). Thus thermal neutron irradiation displayed a much more consistent effect regardless of stage than did the X-rays. CALDECOTT, FROLIK, and MORRIS (1952) in their efforts to demonstrate differences between the effects of thermal neutrons and X-rays on dormant barley seeds found that there was a wide range of height distributions in seedlings grown from X-irradiated seeds; but those grown from seeds exposed to thermal neutrons were remarkably uniform. These results would seem to indicate either that different factors control the sensitivity to the two kinds of radiation or that if the same factors are responsible, they are effective to different degrees. The latter is supported by recent work of CALDECOTT (1954) who found that modification of the water content of barley seeds has a much greater effect on sensitivity to X-rays than to thermal neutrons.

DISCUSSION

The direct correlation between rejoining and fragmentation following X-irradiation of pachytene, diplotene, and early interphase chromosomes was expected since the opportunities for rearrangements to take place increase as the number of breaks increases. Yet with thermal neutron irradiation, zygotene, pachytene, first metaphase, and early interphase chromosomes rejoined at a more or less constant rate regardless of the number of chromosome fragments. This regularity of rejoining would not be expected with little or no fragmentation if the constancy were due to a fixed rate of rejoining or to a saturation-like effect.

An over-all similarity in response to both types of radiation is depicted in the ratios of fragments to rejoins at the various stages tested (fig. 4). Diplotene chromosomes are comparably sensitive, and seem to have a greater ability to rejoin than chromosomes irradiated at other stages. Several other pieces of evidence point to the view that the diplotene nucleus is extremely sensitive and is unable to tolerate even relatively small doses of radiation. Various spindle disturbances occur at low dosages as witnessed by the many polyploid microspores formed and by the cells containing unbalanced genomes seen following irradiation of this stage (SPARROW and SPARROW 1954). Moreover diplotene is the only stage which has never been found to survive a dose of 100r (unpublished data). Actually, diplotene breakage sensitivity may be somewhat higher than that reported, for with the exception of the unbalanced genomes, the cells which survived and were analyzed may have been those which received relatively light injury. Here again, more information is needed before definite conclusions can be reached.

First metaphase also showed relatively high fragmentation-rejoining ratios obtained from irradiation with both X-rays and thermal neutrons. Rejoining may have been impaired to a greater extent following exposure of this particular stage by excessive damage to the broken chromosome ends, making it more difficult for them to reunite with other fragments, i.e. the greater number of unjoinable breaks at first metaphase following thermal neutron irradiation may be in part attributable to a more highly damaging property of the alpha particles and recoil protons produced as previously suggested by CONGER and GILES (1950). However, data from other stages do not support this proposal.

Hypotheses considered to explain results

The hypothesis of BOZEMAN and METZ (1949) that the apparent differences in radiosensitivity found in maturing Sciara oöcytes may be due to varying degrees of "healing" of chromosomes after irradiation, dependent on the interval between exposure time and a healing period, does not explain all the facts found in the present study. First of all, they proposed that rearrangements occured only at interphase when the chromonemata are long and slender, but it can be seen from the data presented above that during at least two periods of the nuclear cycle of Trillium "healing" (reunion) is facilitated. These two periods are meiotic prophase (e.g. diplotene) and microspore interphase. BOZEMAN and METZ also postulated that an insulating material or matrix, which protects and strengthens the chromonema, is renewed in the Sciara oöcyte shortly before and during the prophase movement which may explain the lack of rearrangements following exposure to irradiation during the pre-prophase period. Aberrations would appear as a result of irradiation of meiotic stages since the damaged matrix would not be renewed or produced at least until the interphase between the two meiotic divisions. The evidence presented in this paper, however, clearly indicates that matrix repair as a result of the passage of the cell through interphase is not required in Trillium since a significant number of reunions occur in the same cell generation following meiotic prophase (e.g. diplotene) irradiation.

Results obtained by WOLFF and ATWOOD (1954) using *Vicia faba* confirm those obtained with Trillium as regards the apparent independent nature of the processes of breakage and reunion. In addition to this conclusion, it has been postulated by them that the delay in reunion noted after X-irradiation of the root tips may be due to the radiosensitivity of the process governing DNA synthesis. An hypothesis has been also presented by WOLFF (1954) proposing that rejoining is the result of enzymatic activity which either directly repaired the two broken ends in the form of a rejoin or indirectly provided the energy necessary for such union.

SPARROW, MOSES and STEELE (1952) had postulated a correlation between sensitivity and the time of synthesis of at least one of the three major components of chromosomes, DNA, PNA, and protein. Their results indicated that while the amount of DNA increases per nucleus during prophase when sensitivity is rising, synthesis of DNA is completed by diplotene when the peak of sensitivity is reached. There seemed then to be no decline in DNA content when primary breakage was reduced making the general relationship between the amount of DNA per cell and the radiosensitivity of a stage a dubious association. Also, quantitative cytochem-

ical data on irradiated meiotic Trillium cells indicate that little or no degradation of DNA occurred in the intact cell even after 20,000r of X-rays (SPARROW, MOSES and DUBOW 1952).

The evidence for a definite relationship between DNA content and radiosensitivity of different stages of the nuclear cycle is doubtful even with other biological material. Although *Vicia faba* is most sensitive at late interphase, DNA synthesis as demonstrated by P^{32} uptake occurs earlier and does not coincide with the period of greatest sensitivity (HOWARD and PELC 1953). These authors also found that while 140r of X-rays caused mitotic delay, cells which were in or near the period of DNA synthesis were not impeded in DNA activity. They concluded that division delay is not directly caused by a delay in DNA synthesis.

It has been postulated by SPARROW, MOSES, and DUBOW (1952) that since the protein component is considered to be required to maintain the structural continuity of the chromosome (MIRSKY and RIS 1951; KAUFMANN *et al.* 1951), that chromosome breakage might involve not only DNA damage but also alteration in the protein component of the chromosome. It is therefore suggested that repair of the nucleoprotein would necessarily be concomitant with the rejoining process and that perhaps stages of relatively high rejoining such as diplotene and interphase are characterized by periods of protein synthesis. It has in fact been found in Tradescantia that during late meiotic prophase and at microspore interphase there is a sudden manifestation of metabolism in the phosphoprotein and ribonucleic acid components of the cell (TAYLOR 1953; MOSES and TAYLOR in press). It may be that while these components are actively metabolic they tend to promote "healing" (rejoining). Likewise since ionizing radiations are known to interfere with both DNA and protein metabolism (PELC and HOWARD 1952; HEVESY 1952; FEINSTEIN and BUTLER 1952; ALTMAN *et al.* 1949) then irradiation of certain stages may interfere with the process of reunion.

The above proposals should not be considered as definitive. Undoubtedly correlations will ultimately be made between radiosensitivity and physiological or cytochemical conditions within the cell, but the present status of our knowledge in this area leaves much to be desired. However, two further points seem to warrant brief consideration. The first related to the nucleolar cycle and the second to the proposed particulate nature of the chromosome as revealed by recent chemical procedures developed by MAZIA (1954). In Trillium the nucleolus is known to disappear at late meiotic prophase and reappear during second telophase or early microspore interphase. It has been suggested that the nucleolar PNA may shift to the chromosomes during late prophase and back into the nucleolus when it reforms at telophase (JACOBSEN and WEBB 1952). Since these periods correspond approximately with the two periods of high rejoining (diplotene and early interphase) a relationship of unknown nature would seem plausible.

Recent evidence suggests that chromosomes may be composed of submicroscopic particulates bound together by Ca or Mg (BERNSTEIN and MAZIA 1953; MAZIA 1954). Experimentally, chromosomes may be dispersed if Ca or Mg ions are removed or bound and a low ionic strength exists (MAZIA 1954). If the phenomenon of chromosome breakage is related to splitting of Ca or Mg bonds rather than to a direct

effect on the protein component as suggested above, it is conceivable that physiological changes at certain stages in the nuclear cycle might in turn be reflected in the ease with which chromosome breaks are induced. STEFFENSEN (1953, 1955) has presented evidence that Ca and Mg deficiencies do increase both spontaneous and radiation induced breakage. He has also noted that these deficiencies cause considerable chromosome stickiness. It seems reasonable, therefore, that physiological changes influencing the state of the macromolecular binding by Ca, Mg or both could effect the phenomenon of rejoining as well as primary breakage.

Since no direct relationship between fragmentation and rejoining exists, it appears that fragmentation and rejoining are governed by two separate mechanisms. If, however, they are controlled by the same mechanisms, they must be independent as far as their rates of reaction are concerned. Regardless of the verity of the hypotheses concerning these differences in radiosensitivity between stages and between fragmentation and rejoining, it has been shown that sensitivity to fragmentation is not necessarily related to the frequency of rejoining.

SUMMARY

1. Various stages of microsporogenesis in *Trillium erectum* L. were irradiated with 50r of X-rays and ten minutes of thermal neutrons at 4–7 $\times$ 10^8 thermal neutrons per cm^2 per sec. Analyses at first meiotic anaphase and microspore anaphase were made to establish whether a relationship existed between the amounts of chromosome fragmentation and rejoining. Fragmentation was used as an index of breakage sensitivity and dicentric and ring chromosomes were used as an index of detectable rejoining of radiation induced breaks.

2. To facilitate thermal neutron irradiation, anthers were grown in sterile culture media from meiotic prophase through to binucleate pollen. A suitable substrate for the excised anthers was found to be White's medium plus one percent agar. No significant difference in spontaneous aberration frequency was obtained in anthers cultured artificially on White's medium as compared with those left intact in the flower bud (on the rhizome).

3. First meiotic metaphase, known to be one of the most sensitive stages to fragmentation by X-rays, is also shown to be of high sensitivity to thermal neutrons. Microspore interphase, one of the least sensitive stages to X-rays, is likewise found to have a low sensitivity to thermal neutrons. These results indicate that during the nuclear cycle in Trillium there is a general similarity in the rise and fall of chromosomal radiosensitivity to the two radiations.

4. First meiotic metaphase cells were consistently highly sensitive to both thermal neutron and X-irradiation. Relatively little rejoining occurred following irradiation at this stage. The amounts of rejoining and fragmentation varied independently from bud to bud following both X-ray and thermal neutron treatments of first metaphase with the standard doses.

5. With both thermal neutron and X-ray treatment, the amount of rejoining in cells irradiated at diplotene was dependent upon the number of fragments induced.

6. There seems to be no strict dependence of detectable rejoining upon the amount of visible fragmentation caused by either X-rays or thermal neutrons, since stages

showing almost identical amounts of first anaphase fragmentation, namely pachytene and diplotene, are characterized respectively by low and high degrees of rejoining. Similarly the meiotic and microspore scorings following diplotene irradiations showed no significant difference in number of rejoins per 100 cells but did give significantly different fragment counts.

7. After 50r of X-rays chromosome fragmentation varied more than twenty-fold between the most sensitive and the least sensitive stages studied while a dose of ten minutes of thermal neutrons yielded less than a four-fold variation. Rejoining varied by a factor of eight from stage to stage following X-irradiation whereas a factor of four existed after thermal neutron irradiation. Thus thermal neutrons produced a much more uniform effect regardless of the stage irradiated than did the X-rays.

8. Rejoining, calculated as a function of fragmentation, was found to follow the same general trend after both X-ray and thermal neutron irradiation, indicating that the same or closely related indistinguishable mechanisms were damaged by both radiations.

9. The processes of breakage and reunion take place at characteristically varying degrees at different stages in the nuclear cycle and may be controlled by independent mechanisms.

ACKNOWLEDGMENT

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INHERITANCE IN THE GREEN ALGA CHLAMYDOMONAS REINHARDI

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STUDIES of inheritance in the green alga *Chlamydomonas reinhardi* were initiated to provide material for analysis of heritable alterations affecting the chloroplast and other cytoplasmic bodies. In this paper methods are presented for genetical analysis of this organism and the patterns of inheritance of several factors which show 2:2 segregation are described. Data from tetrad analysis are used for the detection of linkage, and to compute the approximate distance of each factor from its centromere. One factor has been found to show only first-division segregation, making available in Chlamydomonas the equivalent of ordered tetrads. These results will be compared with the non-Mendelian inheritance of streptomycin resistance previously reported in this strain (SAGER 1954), and problems in the detection of non-chromosomal inheritance will be discussed.

DESCRIPTION OF THE ORGANISM

The choice of *Chlamydomonas reinhardi* for these studies was based upon a number of criteria. A single-celled microorganism was desired, containing one or more discrete chloroplasts, able to grow in the dark on a reduced carbon source, exhibiting a readily controlled complete sexual cycle, and able to carry out all stages of its life cycle on a chemically defined medium. It was my good fortune to obtain mating type strains of such an organism from PROF. G. M. SMITH of Stanford University.

The first genetic studies with Chlamydomonas were carried out by PASCHER (1916) who intercrossed two strains ("species") and classified the offspring for a number of morphological characters by which the parental strains differed. He pointed out (PASCHER 1918) that recovery of all four products of meiosis provided a direct demonstration of Mendelian segregation. Extensive investigations of inheritance in Chlamydomonas have been reported by MOEWUS (1940) in *C. eugametos* and closely related strains. Many aspects of these data have been evaluated (for summary see SONNEBORN 1951 and RAPER 1952), but as yet none of the work has been independently repeated. Genetic studies by LEWIN (1953) with *C. moewusii* have demonstrated 2:2 segregation of factors affecting flagellar motility, p-amino benzoic acid and thiamine requirement, twinning and mating type. In *C. reinhardi* SMITH and REGNERY (1950) have reported 2:2 segregation of mating type difference; and HARTSHORNE (1953) has reported 2:2 segregation of "eyeless", a factor determining presence or absence of a morphologically detectable eyespot. Linkage in *C. reinhardi* has recently been found by EBERSOLD (cited in PERKINS 1954).

The life cycle of *C. reinhardi* as first described by REINHARD in 1876 under the name *C. pulvisculus* (GOROSCHANKIN 1891) resembles that of other heterothallic,

[1] This work was begun during the tenure of a Merck Fellowship in the Natural Sciences of the Natural Research Council.

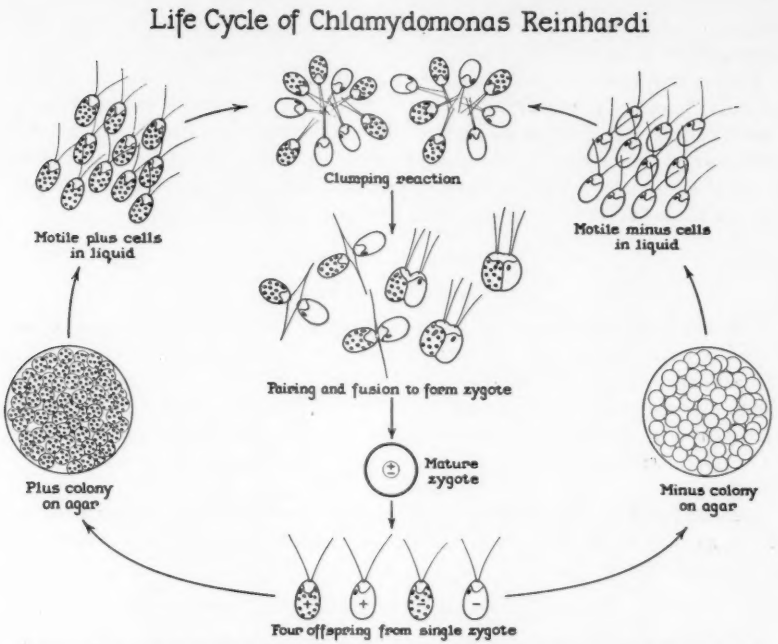
Life Cycle of Chlamydomonas Reinhardi
Clumping reaction
Motile plus cells in liquid
Motile minus cells in liquid
Pairing and fusion to form zygote
Mature zygote
Plus colony on agar
Minus colony on agar
Four offspring from single zygote

FIGURE 1.—The life cycle of *Chlamydomonas reinhardi*, showing the segregation of mating type, denoted by + and −, and of the marker y_1 (the dotted cells are y_1^+, the undotted are y_1^-).

isogamous chlamydomonads (SMITH 1950). It is shown diagrammatically in figure 1. The two mating types, being morphologically undifferentiated, are arbitrarily designated *plus* and *minus*. Clones may be propagated indefinitely in the vegetative state. When the two mating types are mixed under suitable conditions, fusions occur between single cells of opposite mating type, leading to zygote formation. The zygote does not divide vegetatively. After a maturation period it undergoes reduction, giving rise to four zoospores, the haploid products of meiosis.

Although the mating types are isogamous, they can be distinguished by growing the two mating types under different physiological conditions, or by the use of morphological mutants (SAGER and GRANICK 1954). The mating process was studied in crosses in which the parental cultures were distinguished: (1) as green vs. colorless mutant strains, or (2) as deep green cells grown on a high nitrate medium, vs. pale green cells distended with starch grains as a result of growth on a low nitrate medium. Since the mating process was previously described (SAGER and GRANICK 1954), only observations of particular relevance to genetic analysis will be mentioned here.

Mass matings have been followed under the microscope in an attempt to detect copulation between cells of the same mating type in such mixtures. No cytological evidence of "illegitimate copulation" has been found. Rarely, triple fusions have been

observed, but in too low a frequency to affect genetic data. However, one strain has been isolated which regularly produces over 5 percent triple fusions.

Each zygote appears to contain all the contents of the two cells from which it was formed; no loss of protoplasm has been detected when the fusion process was observed under phase contrast microscopy. When the zygote germinates, the cell wall ruptures and four zoospores emerge. Under some conditions, more than four cells are found, due to a mitotic division following meiosis. That these cells do arise from supernumerary mitoses has been demonstrated with genetically marked stocks.

MATERIALS AND METHODS

Growth of the organism

The nutritional requirements of *C. reinhardi* for growth and sexuality (SAGER and GRANICK 1953, 1954) have been reported previously. *C. reinhardi* grows equally well on agar, where single cells give rise to round discrete colonies, and in liquid culture, where cells are kept in uniform suspension by growth in test tubes on a roller tube apparatus. Cultures are maintained routinely in a 25°C room, either in dark cabinets or in fluorescent light of 700–1000 f.c. (General Electric "standard warm white" tubes).

Medium I plus 0.1 percent sodium acetate is used for the maturation and germination of zygotes, which grow poorly on the high-phosphate Medium II (cf. SAGER and GRANICK 1953).

Technique of crossing

Equal numbers of sexually active cells of the two mating types are mixed under conditions assuring over 90% pairing and fusion within about one half-hour (SAGER and GRANICK 1954). The suspension is then diluted and plated on agar, and after five days in the dark at 25°C, the zygotes can be germinated. Individual zygotes are then located with a dissecting microscope and isolated free-hand by means of a special tool: a hypodermic needle (# 23) filed flat at the tip to form a tube and bent at a 90° angle one inch from the tip. An agar plug carrying a single zygote at one end is bored out with the implement and blown onto a fresh agar plate by means of a hypodermic syringe. In this way, zygotes can be isolated with speed and facility. Not only is the zygote isolated, but its new location is indicated by the presence of the agar plug.

Isolation of products of meiosis

Freshly isolated zygotes are germinated in a drop of water, in which the zygote wall cracks and the zoospores become motile (SMITH and REGNERY 1950). When the drop dries down, each swimming cell is immobilized on the agar, where it divides vegetatively, producing an isolated colony. Thus, Chlamydomonas microdissects itself out of the zygote. A petri plate on which three zygotes were germinated is shown in figure 2. Each cluster of four colonies represents the four products of meiosis.

Mutant strains employed

Wild type *C. reinhardi* is green both when grown in the dark and in the light. Strain *21gr* was chosen, following repeated sub-culture in the dark, for its ability to

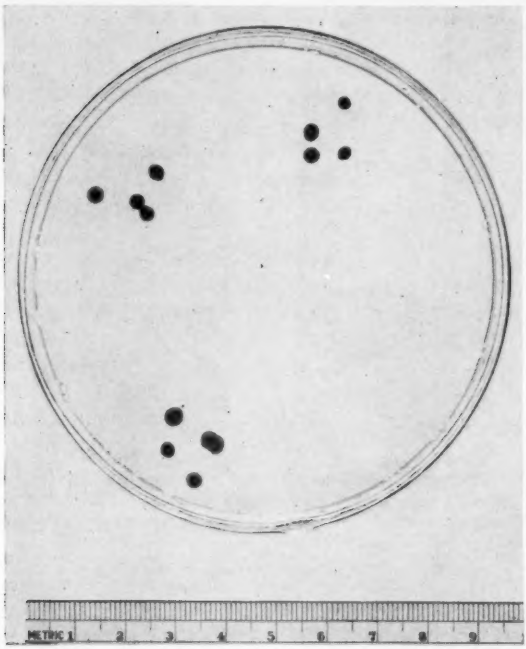

FIGURE 2.—Petri plate on which three zygotes have germinated, each producing four zoospore clones. The agar plug on which the zygote was transferred can be seen in two instances.

grow well in the dark. This single colony isolated from wild type stock (SMITH'S strain 137+) has been the source of all *plus* mating type strains used in this study.

Strain *4y* produces yellow pigments, but little or no chlorophyll, when grown in the dark. When grown in the light, it is indistinguishable from normal green strains. Strain *4y* was recovered as a spontaneous mutant from a normal green strain of *minus* mating type (SMITH's strain 137−). Reversion to green occurs with a low frequency. This isolate has been the source of all *minus* mating type strains used in this study.

Strain *41*, selected following U-V irradiation of *21gr*, is yellow green in both light and dark. Young cultures are yellow, and upon aging some chlorophyll develops. This strain is easily distinguished from *4y* in both light- and dark-grown cultures.

Strain *66* was isolated as a pale green spontaneous mutant from a *21gr* stock culture. In both light and dark it is pale green and requires a reduced carbon source for growth. A few reversions to normal green have been detected.

Strain *164*, selected after U-V irradiation of *21gr*, is yellowish-brown due to a block in chlorophyll synthesis and accumulation of reddish-brown chlorophyll precursors. The presence of protoporphyrin 9 has been established spectrophotometrically. Carotenoid pigments are also present.

Strains *sr-1* and *sr-3* are resistant to streptomycin; they were selected by plating

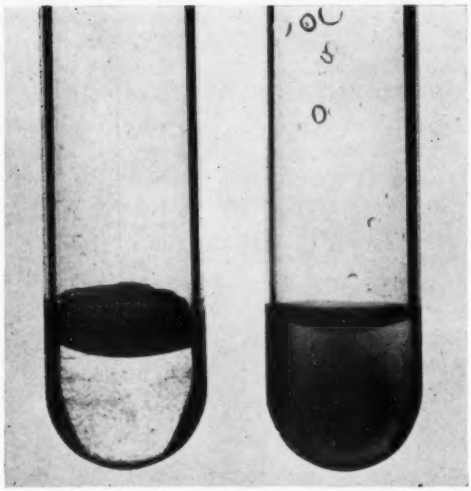

FIGURE 3.—Classification for mating type, based upon the formation of zygote pellicle in tube on the left but not on the right (for details see text).

a suspension of *21gr* onto agar containing 100 μg/ml streptomycin sulfate after ultraviolet irradiation. They are green in the dark, give rise to equal numbers of colonies on agar, with and without 100 μg/ml streptomycin, but are sensitive to higher streptomycin concentrations.

Strains *msr-1* through *msr-6* were selected for resistance to methionine sulfoximine, a competitive inhibitor of methionine (REINER, *et al.* 1950). Strain *4y*, killed by 100 μg/ml of methionine sulfoximine, was plated onto agar containing 500 μg/ml of the compound. The surviving clones were found, on subsequent testing, to be fully resistant to 500 μg/ml of methionine sulfoximine. (The generosity of Wallace and Tiernan Products, Inc., in supplying this compound is gratefully acknowledged.)

Further characterization of these mutant phenotypes will be presented elsewhere. In this paper we shall be concerned solely with their inheritance.

Classification of offspring

Pigment mutants were classified by direct observation of dark-grown cultures. Resistance to streptomycin and to methionine sulfoximine was determined by streaking zoospore clones onto agar with and without 100 μg/ml streptomycin, and 500 μg/ml of methionine sulfoximine, respectively.

A technique has been developed for mating type classification which is much faster and simpler than the methods of MOEWUS (1940), SMITH and REGNERY (1950), and LEWIN (1953). When freshly formed zygotes are left standing in liquid suspension, they aggregate into large stringy clumps, or collect in the meniscus to form a distinct pellicle, as shown in figure 3. This aggregation, due to the stickiness of the secreted capsular material, can not be disrupted by shaking. Vegetative cells

have never been seen to aggregate in this way. Consequently, these characteristic clumps and pellicles provide a simple diagnostic test for mating type. Cells of a clone to be tested are added to suspensions of *plus* and *minus* tester stocks. The result of a typical test is shown in figure 3, in which a pellicle has formed with *plus* but not with *minus* tester, indicating that the unknown clone was of *minus* mating type.

RESULTS

Classification of data

The classification of the progeny from one cross is presented in table 1. It will be noted that the zygote sets do not always contain the same number of clones, although four or eight are most frequently encountered. When alleles affecting growth are segregating, the early post-meiotic divisions may not be synchronous; even the first division after meiosis may be greatly delayed in some genotypes. However, by the use of multiply-marked stocks in this and other crosses, it has been found that no more than four different classes of offspring arise from a single zygote, no matter how many clones are found. Zygote sets consisting of three or more clones have been analyzed in all crosses. In the case of missing clones, or incomplete identification of phenotypes, the additional data have been inferred where possible on the basis of the expectation of 2:2 segregation.

Variability in the frequency of crossing over

The markers used most extensively in crosses to be reported in this paper are y_1 and *mt*. These two markers, one determining the presence (y_1^+) or absence (y_1^-) of chlorophyll in dark-grown cells, and the other determining mating type (mt^+ and mt^-), were both found to segregate 2:2 among the offspring of all zygotes. Figure 4 shows the eight colonies recovered from a single zygote in a cross of $y_1^+mt^+ \times y_1^-mt^-$. The four light colonies are yellow in the dark (y_1^-); the four dark ones are green (y_1^+).

TABLE 1

Classification of offspring from cross of $y_1^+sr_1mt^+ \times y_1^-ss_1mt^-$ (+ + + × − − −)

									y_1/mt	y_1/sr	mt/sr
1851	+ + −	+ + +	− − +	− − −					T†	P†	T
1852	+ + −	+ + −	+ + −	− − +	(additional mixed clones)				NP†	T	T
1853	+ + −	+ − −	+ + −	− + +	− − +	− − +	(+ − −)	(− + +)*	P	T	T
1854	+ − +	+ − +	− + −	− + −	− − −	(− − −)	(+ + +)	(+ + +)	T	P	T
1855	− − +	− − +	− − −	+ + +	+ + −	(+ + −)	(− − −)	(+ + +)	T	NP	T
1856	+ − +	− + +	− + −	− + −	− + +	− + −	+ − +	(+ − −)	T	P	T
1857	+ + +	+ + −	− − +	− − −					T	P	T
1861	− − −	− − −	+ + +	(+ + +)					P	P	P
1862	+ + +	+ + −	+ + −	+ + +	+ + +	− − +	(− − −)		T	P	T
1863	+ − −	+ − −	− + +	− − −	(+ + +)				T	T	P
1864	+ + +	+ + −	− − +	− − −					T	P	T
1865	+ − −	+ − −	− + +	− − +	(+ + −)				NP	T	T
1866	+ − −	+ − +	− + −	(− + +)					T	NP	T

* Parentheses denote missing clones of genotype inferred on basis of 2:2 segregation.

† P = parental ditype; NP = non-parental ditype; T = tetratype.

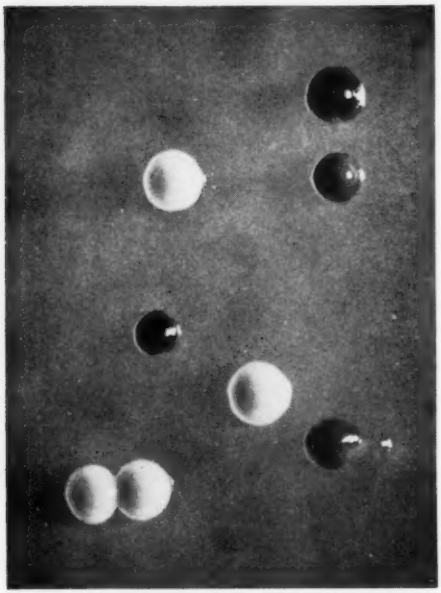

FIGURE 4.—Eight zoospore clones recovered from germination of a zygote heterozygous for y_1. Light colonies are y_1^-; dark are y_1^+.

In table 2, the frequencies are tabulated of the three possible tetrad types for the two markers y_1 and *mt* recovered in a number of crosses. The observed ratio of parental to non-parental ditypes shows that the two factors are unlinked, and the frequency of tetratypes is a function of the second division segregation of both pairs of alleles. It will be shown below that y_1 segregates only at the first division, and therefore, in these data the frequency of tetratypes directly measures second division segregation of *mt*. In only three crosses do the values differ significantly ($P = <0.01$) from the weighted mean. The frequency of second division segregation is changed only from .64 to .61 by excluding these crosses.

Summary of crosses

The results of all crosses made in this study except crosses 1–6 of table 2 are summarized in table 3 in the form of tetrad ratios for all markers classified. (Crosses 1–6 of table 2 were all two-factor crosses involving only y_1 and *mt*.) The difference in total number of tetrads analyzed with respect to different sets of markers in particular crosses results from incomplete classification.

All factors listed in table 3 have shown only 2:2 segregation among the clones of individual zygotes. No more than four classes of offspring have been found in any cross. Segregation of mating type has been classified in 684 zygotes, and no case of homothallism was detected.

TABLE 2

Summary of tetrad ratios for markers y_1/mt *in various crosses*

	Zygote isolation number	P	NP	T	% T	x^2	P
1	5–41	1	2	26	89.7	7.03	<.01
2	410–453	7	9	8	33.3	10.60	<.01
3	636–645	1	1	4	66.6	0.029	>.05
4	705–720	3	3	5	45.4	1.57	>.05
5	898–918	2	7	9	50.0	1.38	>.05
6	919–943	1	4	7	58.4	0.13	>.05
7	510–529	3	8	23	67.5	0.25	>.05
8	650–686 806–827 850–883	2	3	30	85.7	7.31	<.01
9	964 et seq*	7	5	25	66.9	0.26	>.05
10	900–963	8	14	25	53.2	2.20	>.05
11	1012 et seq†	15	15	35	54.0	2.63	>.05
12	1871–1880	1	1	8	80.0	1.11	>.05
13	2277–2290	1	5	8	57.1	0.25	>.05
14	2291–2303	3	0	8	72.7	0.39	>.05
15	2331–2341	1	1	4	66.6	0.03	>.05
16	2481–2495	0	1	3	75.0	0.26	>.05
17	2496–2505	0	1	3	75.0	0.26	>.05
18	2521–2530	1	0	5	83.3	1.04	>.05
19	2721–2738	2	1	9	75.0	0.71	>.05
Total		59	81	245	63.6		
Total excluding crosses 1, 2 & 8		49	67	181	60.9		

* Includes zygotes 964–969; 1038–1044; 1161–1166; 1372–1392; 1851–1880.

† Includes zygotes 1012–1022; 1045–1050; 1094–1110; 1611–1657; 1894–1904; 2061–2077.

In cross # 1 the parental strains were *41*, the original yellow-green mutant clone, and *4y*, which is yellow in the dark. In cross # 2, recombinant offspring of *41* × *4y* were inter-crossed and all four classes were again recovered with similar frequencies.

The pale green mutant, strain *66*, is difficult to keep alive. Only three classes of progeny were recovered from the cross *66* × *4y*, the double mutant $pgr^- y_1^-$ apparently being lethal. No intercrosses with other mutants were attempted. On the other hand, strain *164*, source of the *br* marker, grows well in the dark or in dim light and so do recombinant clones carrying the br^- factor.

The tetratype frequencies for y_1/sr_1 do not differ significantly from those for y_1/sr_3 and, consequently, the data are combined for calculation of centromere distance. The same considerations apply to the data on msr_1 — msr_6. (The streptomycin resistant and methionine sulfoximine resistant strains are called *sr-1*, *msr-1*, etc. The corresponding markers are referred to as sr_1, msr_1, etc.)

Computation of centromere distances

In table 4 the tetratype frequencies from tables 2 and 3 have been summarized. These tetrad ratios have been used for the calculation of second division segregation

TABLE 3

Summary of three-, four-, and five-factor crosses giving P:NP:T frequencies

	ygr/mt	*y₁/ygr*	*br/mt*	*br/y₁*	*sr₁/y₁*	*sr₁/mt*	*msr/sr*	*msr/mt*	*msr/y₁*
1. $y_1^+ygr^-mt^+ \times y_1^-ygr^+mt^-$	3:7:27	18:12:7							
2. $y_1^+ygr^+mt^- \times y_1^-ygr^-mt^+$	4:8:23	15:13:9							
3. $y_1^+pgr^-mt^+ \times y_1^-pgr^+mt^-$		*y₁/pgr* 4:7:19							
4. $br_1^-mt^+ \times br_1^+mt^-$			3:0:7						
5. $y_1^+br_1^-mt^+ \times y_1^-br_1^+mt^-$		*br/ygr*	0:0:6	29:36:18					
6. $br_1^+ygr^-mt^+ \times br_1^-ygr^+mt^-$		14:14:15							
7. $br_1^-ygr^+mt^+ \times br_1^+ygr^-mt^-$	4:1:4	6:4:6	2:2:3						
8. $sr_1mt^+y_1^+ \times ss_1mt^-y_1^-$					15:9:11	4:10:23			
9. $sr_9mt^+y_1^+ \times ss_9mt^-y_1^-$					3:2:5 (sr_3)	1:1:7 (sr_3)			
10. $msr_1y_1^-mt^- \times mss_1y_1^+mt^+$								1:4:15	6:1:15
11. $msr_2y_1^-mt^- \times mss_2y_1^+mt^+$								2:3:7	1:2:8
12. $msr_3y_1^-mt^- \times mss_3y_1^+mt^+$									2:3:7
13. $msr_4y_1^-mt^- \times mss_4y_1^+mt^+$								0:1:5	1:2:3
14. $msr_5ss_1y_1^-mt^- \times mss_5sr_1y_1^+mt^+$					9:7:9	1:2:12	5:3:7	0:0:8	0:1:3
15. $msr_6ss_1y_1^-mt^- \times mss_6sr_1y_1^+mt^+$							1:2:4	1:0:4	0:3:1
16. $ygr^+msr_6sr_1mt^+ \times ygr^-mss_6ss_1mt^-$	2:1:10 *br/sr*	4:1:20 *br/msr*	5:6:12 *br/mt*			1:1:7	4:3:14	1:0:11	
17. $br^+msr_6sr_1mt^+ \times br^-mss_6ss_1mt^-$	5:5:9	4:4:13	1:3:14	*br/y₁*		1:0:14	3:2:10	2:4:10	
18. $y_1^+br^+sr_1msr_9mt^+ \times y_1^-br^-ss_1mss_9mt^-$	2:1:4	0:1:6		4:0:2	3:2:2		0:0:6		0:0:7

TABLE 4

Summary of all tetrad ratios

Markers	P NP T	% T	Markers	P NP T	% T
1. *y₁/mt*	59:81:245	64	9. *msr/mt*	7:12:60	76
2. *y₁/ygr*	33:25: 16	22	10. *msr/ygr*	4: 1:20	80
3. *ygr/mt*	13:17: 64	68	11. *msr/sr*	13:10:41	64
4. *pgr/y₁*	4: 7: 19	63	12. *br/y₁*	33:36:20	23
5. *y₁/sr*	30:20: 27	35	13. *br/mt*	6: 5:30	73
6. *ygr/sr*	5: 6: 12	52	14. *br/ygr*	20:18:21	36
7. *mt/sr*	8:14: 63	74	15. *br/sr*	7: 6:13	50
8. *msr/y₁*	10:12: 44	67	16. *br/msr*	4: 5:19	70

Summary of tetratype frequencies

	y₁	*mt*	*ygr*	*pgr*	*sr*	*msr*
y₁	—					
mt	.64	—				
ygr	.22	.68	—			
pgr	.63	—	—	—		
sr	.35	.74	.52	—	—	
msr	.67	.76	.80	—	.64	—
br	.23	.73	.36	—	.50	.70

frequencies, following the method of PERKINS (1949) and WHITEHOUSE (1950), for three unlinked markers. (It is apparent from the ratio of P:NP ditype tetrads that none of the tested combinations exhibit linkage (PERKINS 1953).) The tetratype frequencies involving *mt*, *pgr*, and *msr* are too large to be used for the initial com-

TABLE 5

Frequencies of second division segregation (computed from data of table 4 by method of WHITEHOUSE *1950)*

	y_1	*ygr*	*br*	*sr*
1. y_1-*ygr*-*br*	.01	.21	.22	—
2. y_1-*sr*-*br*	0	—	.27	.39
3. y_1-*ygr*-*sr*	0	.30	—	.41
4. *ygr*-*sr*-*br*	—	.24	.18	.43

putations. However, the method was applied to the combinations: y_1-*ygr*-*sr*, y_1-*ygr*-*br*, y_1-*sr*-*br*, and *ygr*-*sr*-*br*, with the results listed in table 5.

The most reliable calculation is from the combination y_1-*ygr*-*br* because 50–100 zygotes were analyzed for each of the three tetratype frequency values, and because all three values are fairly low. From this calculation it appears that the marker y_1 segregates entirely or almost entirely at the first division. This is borne out by the small negative values (equivalent to zero) computed for second division segregation of y_1 by using the combinations y_1-*br*-*sr* and y_1-*ygr*-*sr*. However, the latter calculations are less accurate than those for y_1-*ygr*-*br*, since the tetratype frequencies for *br*/*sr* and for *ygr*/*sr* are based on small samples (cf. table 4).

Since y_1 shows only (or almost only) first division segregation, the tetratype frequencies for all markers with reference to y_1 can be considered directly as second division segregation frequencies, and on that basis compared with the computed values. As can be seen in table 5, the second division segregation frequency for *ygr* computed from y_1-*ygr*-*br*, compares closely with the y_1/*ygr* tetratype frequency shown in table 4. The same is true for *br*. In the case of *sr*, the computed value which comes closest to the y_1/*sr* tetratype frequency is from the combination y_1-*br*-*sr*.

The computation method gives only a rough approximation to actual second division segregations because the data are subject to large sampling errors (BARRATT *et al.* 1954). Nonetheless, on the assumption that the markers are chromosomal, the values are useful in providing an approximate measure of centromere distance for each marker.

In table 6, the centromere distances for the six markers are tabulated, the confidence limits for each tetratype frequency having been determined from the curves of BARRATT *et al.* (1954). Uncorrected centromere distances are based on the assumption of complete interference. "Corrected" centromere distances, based on the mapping function derived for Neurospora by BARRATT *et al.* (1954) are also presented as an example of the shift in values to be expected from interference of the intensity found in other organisms.

Independence of markers

That unlinked markers are in fact on different chromosomes can be demonstrated in some cases with tetrad ratios. As pointed out by BARRATT *et al.* an NP:T ratio of more than 1:4 indicates that the two loci involved are independent. This criterion of independence can be applied only to the pairs: y_1/*ml*, y_1/*ygr*, y_1/*sr*, y_1/*br*, and *ygr*/*br*. Thus, y_1 is independent of all other markers except possibly *pgr*

TABLE 6

Centromere distances computed from tetratype frequencies

Marker	% T*	Range**	Centromere distance (uncorrected)	Centromere distance (corrected, $k = 0.2$)†
mt	64	61–68	31–34	35–41
ygr	22	14–31	7–15	7–16
pgr	63	42–80	21–40	23–54
sr_1	35	25–45	12–22	13–24
br	23	14–32	7–16	7–17
msr	67	50–78	25–39	28–51

* %T from table 4, recombination with y_1.

** Range based on P = 0.05.

† Corrected centromere diatance based on tetrad mapping function derived for Neurospora (BARRATT *et al.* 1954).

and *msr*, and *ygr* is independent of *br*. Consequently, at least three linkage groups must be present, but it is not excluded that all markers are independent, in which case the existence of seven groups must be postulated.

DISCUSSION

Relation of markers studied to genetic loci

In initiating genetic analysis of a new organism, it becomes necessary to demonstrate whether the markers whose segregation has been observed, can be equated with the genetic loci of other forms. Unfortunately, no detailed study of the meiotic chromosomes of *C. reinhardi* has been made and so, as yet, the orthodoxy of meiosis in this organism can be inferred only from the genetic data. In view of the regular segregations observed, it has been assumed as a working hypothesis that the behavior of the chromosomes at meiosis in this organism is typical.

The markers dealt with in this study have all shown 2:2 segregation among the offspring of individual zygotes and, consequently, they may be considered to represent alternate pairs of unit factors. Are these unit factors necessarily chromosomal?

A kind of cytoplasmic inheritance can be postulated, involving unit factors, for example unit cytoplasmic organelles. Chlamydomonas contains a single chloroplast, pyrenoid, and eyespot. If one of these organelles were genetically autonomous and mutated directly, then a cross of mutant with wild type might lead to 2:2 segregation simulating chromosomal behavior. Such a cytoplasmic factor could be distinguished from a nuclear gene if it showed no linkage with any chromosome in a well-marked genome. The goal of a well-marked genome is some years away in Chlamydomonas.

At present, nonetheless, a preliminary distinction can be drawn between factors which are more or less likely to be non-chromosomal, based upon their segregation pattern at meiosis.

Non-chromosomal unit factors, in order not to be lost during meiosis, must presumably be reduplicated and segregated in a precisely oriented way. This requirement could be met by regular segregation of the two parental units at the first meiotic division, followed by reduplication of each and subsequent distribution of

one to each of the four offspring. Such a system would result in 100% first division segregation. A similar model can be proposed which would lead to 100% second division segregation. However, the occurrence of a mixture of first and second division segregation would require more elaborate assumptions. On the basis of this reasoning, admittedly speculative, markers which do show intermediate second division segregation frequencies are considered chromosomal.

In other words, the centromere is used as a marker, and partial linkage of any factor with the centromere is taken as a criterion of its chromosomal location. The fact that the six markers *ygr*, *pgr*, *mt*, *msr*, *sr*, and *br* all do exhibit second division segregation frequencies between .22 and .67 leads then to the conclusion that they are probably chromosomal.

In the case of y_1, however, no second division segregation has been found and therefore, by this criterion, the possibility of a non-chromosomal location cannot be distinguished from that of a chromosomal one close to the centromere. The fact that none of the other markers are linked with y_1 also provides no basis for distinguishing between these alternatives. If y_1 is chromosomal, the fact could be established by linkage data. If non-chromosomal, a different approach is required to demonstrate it directly. Consequently, the site of y_1 may remain an open question for some time.

Meanwhile, because it converts the unordered tetrad of Chlamydomonas into the equivalent of an ordered one, y_1 is a valuable tool for genetic analysis of other markers.

Comparison of y_1 with inheritance of sr_2

In the course of this investigation an instance of non-Mendelian inheritance was found which has been separately described (SAGER 1954). The salient facts will be summarized here for purposes of comparison with the 2:2 segregation data of the present study.

A streptomycin-resistant strain was selected which on crossing with a streptomycin-sensitive wild type strain, gave rise exclusively to streptomycin-resistant offspring, despite the 2:2 segregation of the markers y_1 and *mt* also present in the cross. Subsequently, the markers *br*, *ygr*, *msr* and sr_1 were also found to segregate 2:2 in similar crosses. So long as the streptomycin-resistance factor, designated sr_2, was carried by the parent of *plus* mating type, it was transmitted to all offspring, even after four successive generations of backcrossing. However, in most cases, sr_2 was not transmitted by the parent of *minus* mating type.

About 4% of all zygotes examined were exceptional in that a cross of $sr_2mt^- \times ss_2mt^+$ gave 4 resistant:0 sensitive, instead of 0 resistant:4 sensitive; that is full transmission instead of none at all. The further progeny of these exceptional tetrads were not themselves exceptional, but transmitted the streptomycin-resistance property in the original manner; that is through the mating type *plus* parent.

In view of the possibility that y_1 may be non-chromosomal, it is of some interest to compare its inheritance with that of sr_2. No difference has been observed in the transmission of y_1 through the *plus* and *minus* parental types, and no correlation has been observed in the inheritance of y_1 and of sr_2. Thus, whatever the physical basis of inheritance for these two factors, they differ in the mechanism of transmission.

SUMMARY

1. Methods have been developed for the study of inheritance in the isogamous, heterothallic green alga, *Chlamydomonas reinhardi*.

2. Segregation ratios have been determined for seven pairs of characters, each of which has shown only 2:2 segregation in individual tetrads, indicating unit factor inheritance. The characters studied were mating type and the mutant phenotypes: resistance to streptomycin, resistance to methionine sulfoximine, yellow, yellow-green, pale green and brown.

3. These markers were tested for recombination in 16 out of the 21 possible combinations and no instance of linkage was detected.

4. The map distance of each marker from its centromere was determined by computation from tetratype frequencies.

5. The marker y_1 was found to segregate only at the first division, providing a tool by which the unordered tetrad of Chlamydomonas could be treated as if ordered, and the second division segregation frequencies of the other markers determined directly.

6. A criterion of chromosomal location of individual markers based on centromere linkage was applied to the seven markers of this study. On this basis, all have been considered chromosomal except y_1, the location of which cannot be determined from available data.

7. The possibility was raised that y_1 may be non-chromosomal, but its mode of inheritance differs in important respects from that of the previously reported non-Mendelian factor for streptomycin-resistance, sr_2.

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SEX-LINKED LETHAL MUTATIONS INDUCED BY THERMAL NEUTRONS IN MALE AND FEMALE DROSOPHILA MELANOGASTER[1]

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THE experiments to be described are concerned with a comparative study of the mutagenic effectiveness of thermal neutrons in producing sex-linked lethal mutations in male and female *Drosophila melanogaster*. Some of this work has appeared previously in abstract form (KING 1952a). Preliminary comparative studies of the mutagenic effectiveness of X-rays in producing sex-linked lethal mutations in male and female Drosophila have already been described (KING 1952b). The most extensive study to date on cytogenetic changes accompanying thermal neutron treatment is that of CONGER and GILES (1950) working with Tradescantia.

PHYSICAL METHODS

The thermal neutrons used in the experiments were produced in the thermal column of the Brookhaven nuclear reactor by moderation of fast neutrons from uranium fission by elastic collision with graphite. The exposure chamber is a 10 inch cubical space at the center of a 5 foot cube of graphite. Exposures are made in a lucite box having a cubical volume of eight inches on a side. The thermal neutron flux expressed as the number of neutrons crossing a surface area of one square centimeter per unit time is calculated from measurement of the induced radioactivity of gold foils exposed with the experimental material. The slow neutron flux in the thermal column varies linearly with pile power. During our experiments it amounted to $3 \times 10^{12} n_{th}/cm^2/hr$. The cadmium ratio is about 10^4 and the gamma ray contamination 125r/hr $\pm$ 30%. The temperature in the thermal column varies between 21 and 25°C.

For exposure in the thermal column groups of 100 male or female Drosophila are placed in small lucite chambers (outer dimensions $1\frac{3}{4} \times 1\frac{3}{4} \times \frac{1}{2}$ inches). These chambers consist of a lucite base and overlapping cover. The base is a rectangular frame $\frac{3}{8}$ inches high and $\frac{3}{16}$ inches thick enclosing a space $1\frac{1}{8} \times 1\frac{1}{8} \times \frac{3}{8}$ inches. The bottom of the frame is covered by a 0.003 inch (7.72 mg/cm²) polystyrene film. The inner walls of the frame are generally covered with a 1 mm thick layer of fly medium. In such containers flies can be maintained in a healthy condition for treatments of 72 hours or more, while flies often die from desiccation within 4 hours in food-free chambers. The covering frame holds taut a layer of cheese cloth over the top of the base frame, and thus flies are prevented from escaping. Drosophila are etherized through the cheese cloth. Polystyrene film (0.003 inch) glued over the top

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of the covering frame reduces evaporation and prevents the food within from drying out during long exposures. The floor of the thermal column holds a maximum of sixteen chambers at any one time.

BIOLOGICAL METHODS

The genetic setup used is a slight modification of that outlined by MULLER (1952), who has kindly supplied us with the necessary stocks for synthesis of the breeding systems used. The method has two advantages over the standard "M-5" technique. First, great numbers of the females used in the P_1 radiation crosses may be obtained with a minimum of effort, since these females are "automatic virgins". Second, the collection of lethals produced in the female germ line is facilitated, since lethals detected in the F_2 generation are balanced. The method makes use of modified X-Y conditions where the long or short arms of the Y (represented symbolically as Y^L or Y^S) have become attached to a region of the X chromosome to the right of the centromere. The remainder of the Y is present as a fragment, either a free short arm (Y^S), a free closed long arm (Y^{LC}), or the free long or short arm carrying the left distal region of the X containing the wild type allele of yellow (y^+ *sc*. Y^L or y^+ sc^{VI}. Y^S).

It has been known since the classical studies of BRIDGES (1916), STERN (1929) and NEUHAUS (1939) that males lacking a whole Y-chromosome or either arm of the Y are sterile. SHEN (1932) showed Y-deficient males produce immobile sperm which later degenerate, while STERN and HADORN (1938) demonstrated that testis transplants from Y-deficient males to normal males behaved autonomously, as did reciprocal transplants.

The diagram in figure 1 shows the type of breeding system used. Five stocks are kept which produce "B" males, "D" males, "E" males, "F" males, and "G" males. These are *y w*. Y^S/y^+ *sc*. Y^L and *y f*: = $/y^+$ *sc*. Y^L, *y v f*. Y^L/y^+ sc^{VI}. Y^S and *y f*: = $/y^+$ sc^{VI}. Y^S, *y In 49 v B*.Y^L/y^+ sc^{VI}. Y^S and *y f*: = $/y^+$ sc^{VI}.Y^S, *y In 49 v f*. Y^S/Y^{LC}, and *y In 49 v B*.Y^L/Y^S and *y f*: = $/Y^S$, respectively.

The tandem attached-X chromosome, *y f*: =, has been described by MULLER (1943). The cycling setup shown in the top of the diagram produces "A" and "C" females which are "automatic virgins", since their brothers are sterile. These females serve in the radiation experiments as shown in the lower part of the diagram. The "A" females are mates for treated "B" males and the "C" females are treated and mated to "E" males. In the case of "B", "D" and "E" males, these are always non-yellow, and the presence of yellow males indicates the loss of the Y fragment and the possible presence of a normal Y. This condition enables one to continually check whether or not the breeding system is behaving satisfactorily and to discard aberrant cultures. Patroclinous males are readily observed in the F_1 of all crosses since P_1 males differ in phenotype from F_1 males. "G" and "F" males do not contain the wild type allele of *y*, since the presence of the left hand tip of the X on the Y-fragment would mask mutation occurring in this region when the F_2 was observed. It should be noted that in the case of "F" males crossing over between the X and Y will produce dicentric chromosomes and consequently the stock will be a relatively stable one.

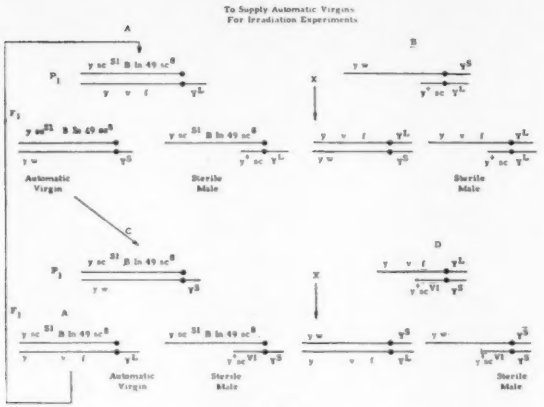

FIG. 1A

FIGURE 1.—The genetic system employed. Modified after MULLER's *Binscy* technique.

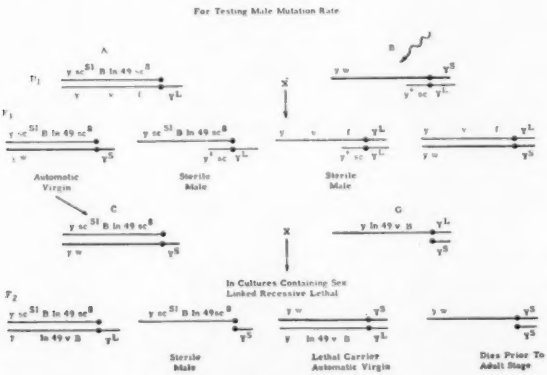

FIG. 1B

As a check on the above setup to see if "A" and "C" females were really automatic virgins *y sc*^{S1} *B In 49 sc.*^{S}/ *y*$^{+}$ *sc.* Y^{L}, *y v f.* Y^{L} / *y*$^{+}$ *sc.*Y^{L}, *y sc*^{S1} *B In 49 sc*^{S} / *y*$^{+}$ *sc*^{VI}.Y^{S}, and *y w.* Y^{S} / *y*$^{+}$ *sc*^{VI}. Y^{S} males were mated singly in creamers to 176, 106, 60, and 72 virgin *bw*; *e* females, respectively. The virgin *bw*; *e* females were obtained from the cross $X.Y^{S}/Y^{LC}$; *bw*; *e* males with *bw*; *e* females. The cultures produced no F_1 flies although many eggs were laid in all cultures.

It should be noted that in the male and female series the *y w.* Y^{S} chromosomes are balanced by chromosomes which differ structurally (*y sc*^{S1} *B In 49 sc*^{S} and *y In 49 v B.*Y^{L}). The Binscy (*y sc*^{S1} *B In 49 sc*^{S}) chromosome is similar to the M5 or Basc (*sc*^{S1} *B In S apr sc*^{S}) chromosome in general use today, differing mainly in that *In 49* is longer than *In S*. Crossing over is reduced to a greater extent in double inversion

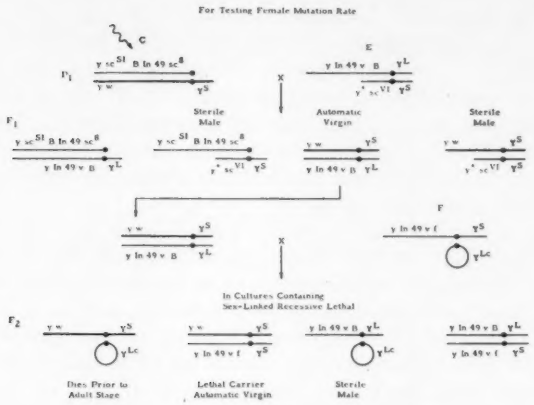

FIG. 1C

heterozygotes than in single inversion heterozygotes, and one should expect recovery of crossover products in the F_2 of the female series. These will include single crossovers (between *w* and *In 49* and B^+ and *In 49*) and double crossovers (between *w* and B^+). For example, a chromosome bearing a lethal located near *B* will yield crossover products including *y w v B* and *y w* v^+ *B*. Here the lethal still would be detected, since white Bar males obviously would be crossover classes. Double crossover class *y w v* B^+ would be viable in the case of X chromosomes bearing centrally located lethals and would be mistaken for the noncrossover class *y w*. However, it is assumed that this class, because of its low frequency of occurrence, will not introduce an appreciable error in mutation rate determinations.

THERMAL NEUTRON TREATMENT

Zero to five day old male and zero to four day old female *Drosophila melanogaster* with the identical X chromosome, *y w*. Y^S, were irradiated under identical conditions in the thermal column for varying periods of time. In the case of males it was found that 48 and 72 hour exposures (amounting to 134×10^{12} and 210×10^{12} n_{th}/cm^2 under the conditions of the experiment) produced sterile flies and consequently exposures were restricted to 24 hours or less. Six exposures of males and three of females were made. The flies were treated in groups of 100 each. In the male series 100 flies were treated at each of the three lowest exposures, 200 flies at the next two exposures and 300 at the highest exposure. In the female series 100, 200, and 300 individuals were treated respectively at the three increasing dosages. Irradiated males were mated with *y* sc^{81} *B In 49* sc^8/y *v f*.Y^L females in half pint bottles, ten pairs per bottle, and the parents removed and discarded on the tenth day. Irradiated females were mated with *y In 49 v B*.Y^L/Y^S males in a similar fashion, and then transferred to fresh bottles every second day for a total of five transfers. After the last transfer all the flies were removed from the bottles and discarded. The F_1 progeny from the five series of cultures thus represent eggs laid by treated females 0–2, 2–4, 4–6, 6–8, and

8–10 days after irradiation. These successive batches of eggs were at increasingly early stages of oögenesis at the time of exposure to thermal neutrons.

In the control series the sperm from 120 "B" males were tested as shown in figure 1. In the female control the mutation rate in the *y w.* Y^S chromosomes from 210 females was tested as shown in figure 1. Here, however, the P_1 flies were allowed to remain in their culture bottles for ten days and were discarded on the eleventh. This means that the control rate is for eggs laid 0–10 days after treatment and is not broken down as in the treated series.

In all cases when the F_1 generation appeared, *y* heterozygous B females were allowed to mate freely for 24 hours in groups of 250–300 with 50 "F" males (female series) or "G" males (male series). Half pint milk bottles with 50 cc of "enriched" Drosophila medium served as mating bottles. (Formula (g component/1000g medium) propionic acid 3.8, agar 8.5, rolled oats 17.0, Anheuser Busch strain G primary, dried yeast 20.8, Karo white corn syrup 58.4, Grandma's molasses 58.6, yellow cornmeal 68.0, water 764.9. This medium remains free of mold for two weeks or more even if dispensed into unautoclaved containers. Growth of Drosophila on the medium is excellent.) Each fertilized female was then transferred to a separate culture bottle where it produced a second generation.

Three-quarter ounce creamers and 1 ounce Dixie sampling and restaurant cups containing 10–15 cc Drosophila medium served as F_2 cultures. The Dixie cups were covered by transparent acetate caps $1\frac{9}{16}$ inches in diameter and 0.03 inch thick. These containers had the advantage of being expendable and were discarded at the end of each experiment, while the acetate caps were saved.

The progeny of each F_1 female was examined through the side of the creamer or the top of the Dixie cup under a dissecting microscope. Magnifications of 6, 9, 10 or 12 × were used. The absence of white-eyed males in cultures containing a minimum of 10 males was generally taken as a criterion for the presence of a sex-linked recessive lethal mutation. Records were kept of the phenotype and number of individuals in each of the lethal cultures. Cases with no white males, less than 10 non-white males, but more than 30 females were classified as lethals. There were only a few of these cases, however.

RESULTS

The experimental data are shown in table 1 and figure 2. It is evident that very few F_1 test females were obtained from the 0–2 and 2–4 day groups although 100, 200, and 300 mature females, respectively, were laying eggs in the three increasing dosage series. All the available F_1 females were tested from the 0–2 and 2–4 day groups, whereas only a portion of the F_1 *y* heterozygous B females from the later groups were tested due to their large numbers. A similar rise in the productivity of X-irradiated females five days after treatment has been reported (KING 1952b).

The thermal neutron dose/mutation frequency relation for each gamete sample is assumed to be linear, and the lines of best fit were computed. The dose action curve for sperm computed from the seven experimental points is given by the equation $y = 0.003 + 1.49 \times 10^{-15} x$. The five dose action curves for the various egg samples were forced through the origin, since the control sex-linked recessive lethal frequency

TABLE 1

Dose-mutation frequency data for different gamete samples

	Thermal neutron exposure (n_{th}/cm^2)	Days between exposure and collection of gamete sample	Lethal frequency	Total X chromosomes tested
Sperm	0	0–10	0.0011	3623
	5.7×10^{12}	0–10	0.0099	1021
	15.6	0–10	0.0316	2439
	19.9	0–10	0.0344	1746
	33.7	0–10	0.0442	1356
	49.5	0–10	0.0801	936
	65.6	0–10	0.0992	534
Eggs	0	0–10	0.0003	6437
	16.6×10^{12}	0–2	0.0264	189
	33.2	0–2	0.0287	174
	49.8	0–2	0.1087	46
	16.6	2–4	0.0171	468
	33.2	2–4	0.0492	244
	49.8	2–4	0.0387	362
	16.6	4–6	0.0160	499
	33.2	4–6	0.0380	764
	49.8	4–6	0.0629	429
	16.6	6–8	0.0188	532
	33.2	6–8	0.0275	763
	49.8	6–8	0.0374	642
	16.6	8–10	0.0032	618
	33.2	8–10	0.0086	818
	49.8	8–10	0.0211	759
				25,399

was very low (0.0003). A standard analysis of variance revealed three facts about the egg data: (1) the difference in the regression lines for the pooled 0–6 day and 6–10 day egg data is very highly significant; (2) the regression line for the pooled egg data for 0–6 days gives as good a fit as the three separate regression lines; and (3) separate regression lines for 6–8 and 8–10 day egg samples give a significantly better fit for the data than does the regression for the pooled 6–10 day sample.

The mutation rates for the different gamete samples are given in table 2. The data indicate that the sex-linked lethal mutation rate for viable eggs laid after exposure to thermal neutrons remains fairly constant for the first six days. Subsequently the rate decreases. Eggs laid 6–8 and 8–10 days following exposure have mutation rates 70% and 30% the original value.

Patroclinous *y v B* males appeared in many F_1 populations of the 0–2, 2–4 and 4–6 day female series. A decrease in the frequency of patroclinous males was noted in the 6–8 and 8–10 day series. Such males may be produced by fertilization of an X-deficient egg by X-bearing sperm resulting in an X0, sterile male. Patroclinous males may also arise by union of a *y In 49 v B*.Y^L-bearing sperm with an egg bearing

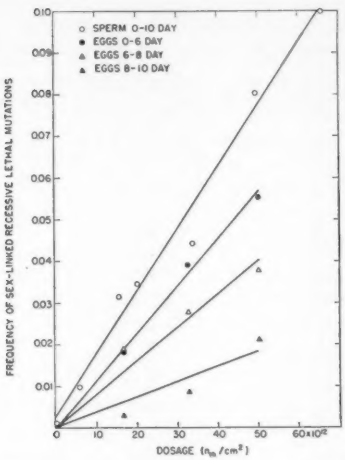

FIGURE 2.—The relation between thermal neutron dose and lethal mutation frequency in surviving X chromosomes for various gamete samples.

TABLE 2

Thermal neutron-induced mutation rates for different gamete samples from Drosophila melanogaster

Gamete	Days between exposure and collection of gamete sample	Mutation frequency per thermal neutron
Sperm	0–10	1.49×10^{-15}
Eggs	0–6	1.13×10^{-15}
	6–8	0.80×10^{-15}
	8–10	0.36×10^{-15}

the short arm of the Y resulting from radiation-induced breakage of the *y w*.Y^S chromosome in the proximal region of the X. Such males would be fertile. The fact that a second generation appeared in cultures and that the F_2 population contained *y v B* females supports the conclusion that some patroclinous males were fertile.

In the male series *y v f* males were found, and some of these were undoubtedly X0 individuals. However, a second generation containing *y v f* females was produced in cultures where all males were supposedly sterile. This means that some of the *y v f* males were fertile and indicates the fertilization of *y v f*.Y^L-bearing eggs by Y^S-bearing sperm. Thus the treatment given the males and females produced fragmentation of the *y w*.Y^S chromosome as well as its complete loss. A sterile F_1 male phenotypically *y* heterozygous *B* was probably of the genotype *y* sc^{81} *B In 49* sc^8/B^+ .Y^S.

DISCUSSION AND CONCLUSIONS

From the thermal neutron dose/mutation frequency relation for sperm one can calculate that a thermal neutron exposure of $1 \times 10^{13} n_{th}/cm^2$ produces 1.8% sex-linked lethal mutations. Substitution of this value in the X-ray dose/mutation fre-

quency relation determined by SPENCER and STERN (1948) yields a value of 740r. Thus 740r of X-rays and $1 \times 10^{13} n_{th}/cm^2$ produce equivalent mutation frequencies. Data are available (KING 1954a) which allow calculation of the radiation dose to Drosophila tissue accompanying exposure to $1 \times 10^{13} n_{th}/cm^2$. With respect to radiations of high specific ionization the $N^{14}(\eta, p)C^{14}$ reaction yields 293 rep of protons and 23 rep of recoiling carbon nuclei, while the $H^1(\eta, \gamma)H^2$ reaction yields 5 rep of deuterons. With respect to sparsely ionizing radiations, 7 rep of 2.23 Mev maximum energy gamma rays ($H^1(\eta, \gamma)H^2$ reaction) are produced and a dose of 430 rep is delivered by 5 Mev maximum energy gamma rays which contaminate the thermal column. If, as has been shown for another radiation of low specific ionization (KING 1954b), these hard γ-rays are 60% as efficient as X-rays in producing sex-linked lethals, then the densely ionizing radiations must be roughly 1.5 times as effective mutagenically as X-rays to account for the observed mutation rates.

The radiation doses delivered to Drosophila tissue by the $Li^6(\eta, \alpha)H^3$ and $B^{10}(\eta, \alpha)Li^7$ reactions are omitted in the above calculations. These reactions are believed to be unimportant, since treatments which increase the average lithium and boron contents of Drosophila tissue by factors of 2 and 100 respectively, are ineffective in increasing the thermal neutron-induced mutation rate (KING 1952a, 1954a).

The yield of mutations does not vary for equal radiation doses delivered at different intensities. It is assumed, therefore, that the large differences in the length of time necessary for delivery of mutagenically effective thermal neutron exposures as opposed to X-ray exposures does not introduce a significant variable into the experiments described above.

It seems unlikely at first thought that the decline in the sex linked lethal rate in successive batches of eggs can be due to selection of lethal-free eggs during oögenesis, since the developing eggs are diploid and the lethal mutations are recessive. It may be, however, that fewer viable restitutions of broken chromosomes occur in pre-meiotic and early meiotic stages than in late meiotic stages and consequently many potential recessive lethals are drawn into chromosome configurations which function as dominant lethals. It is known that oögonia are very sensitive to X-irradiation (BUCHER 1951) and that few gross chromosomal aberrations are recovered from the eggs of irradiated females (SHAPIRO and NEUHAUS 1933; GLASS 1940). If elimination of potential recessive lethals does occur then one might expect to find the reverse of what actually happens, namely, a decrease in the productivity of irradiated females as the mutation frequency in successive batches of eggs decreases. However, this lowered productivity might be masked, if the unaffected oogonia multiply rapidly enough. A second explanation for the lower mutation rates observed in pre-meiotic and early meiotic stages is that the mutation process actually occurs at a lower frequency in these less mature cells because of physiological differences between these and more mature nuclei.

The above calculations depend upon the assumption that the nitrogen concentration per unit weight of Drosophila gonad is the same as the mean nitrogen concentration per unit weight of fly. The difference in the mutation rates induced in the male and female germ line may point to a difference in mutability between sperm and egg chromosomes during late stages of gametogenesis or may simply be due to differences in nitrogen concentration between the male and female gonad.

SUMMARY

A study was made of the sex-linked lethal mutation rates induced in the germinal tissue of *Drosophila melanogaster* males and females by thermal neutrons. Nitrogen capture protons (which are primarily responsible for the biological effects of thermal neutrons in the fruit fly) are approximately 1.5 times as effective in producing sex-linked lethal mutations in sperm as are 90 kv X-rays. Over the range of doses used the lethal mutation rate/dosage relation for X chromosomes of sperm appears to be linear. The mutation rate detected in viable eggs laid following treatment remains fairly constant for the first six days. Eggs laid 6–8 and 8–10 days following exposure have mutation rates 70% and 30% the original value. This original rate is only 75% the rate for sperm. The difference in the mutation rates induced in the male and female germ line may be due to a difference in mutability between sperm and egg chromosomes treated in late stages of gametogenesis or to differences in the nitrogen concentration between the male and female gonad. In the female germ line the lower frequency of mutations recovered from pre-meiotic and early meiotic stages than from late meiotic stages may mean that the mutation process occurs at a lower frequency in less mature cells or that a larger fraction of the potential lethal mutations are drawn off into inviable chromosome recombinations. Exposure of males and females to thermal neutrons also produces loss and fragmentation of X chromosomes in germ cells.

ACKNOWLEDGMENTS

The authors are grateful for the conscientious assistance performed by MRS. AUDREY D. AGNEW and MRS. JEAN B. DARROW. MR. JOEL HENKEL ran the thermal neutron exposures and determined the dosages administered. DR. PHELPS CRUMP outlined the statistical methods used. DRS. I. H. HERSKOWITZ and H. B. GLASS kindly read and criticised the first draft of this paper.

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TRANSLOCATIONS IN MAIZE INVOLVING CHROMOSOME 4

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THE fourth longest chromosome in maize was first shown to carry genes in linkage group 4 by the association of semisterility due to translocation *4-8a* with the *Su su* (sugary endosperm-1) locus and by the normal ratios for *Su su* found by MCCLINTOCK (unpublished, see EMERSON *et al.* 1935) in plants trisomic for chromosome 8. The chromosome 4 translocation data summarized in the following pages amply confirm these results.

Sugary endosperms can be easily and rapidly classified, and endosperm-marked translocations in which the point of interchange shows little recombination with sugary are likely to prove valuable in a great many types of studies as ANDERSON (1952) pointed out in a case where *Y* (yellow endosperm-1) on chromosome 6 was closely linked with *su* through the use of *T4-6a*. Since, as a prerequisite to such studies, it is necessary to know the position of the interchange points relative to the gene loci under investigation, extensive studies with four genes Ts_5 (tassel seed-5) *su*, *Tu* (tunicate ear) and gl_3 (glossy seedling-3) are summarized here. EMERSON, BEADLE, and FRASER (1935) give the map positions for these genes and ANDERSON and RANDOLPH (1945) and ROMAN (1947) have shown that *su* is on the short arm to the left of the centromere. The partial linkage map thus is:

Ts_5	*su*	*Tu*	gl_3
56	71	100	111

At midprophase I of meiosis in microspore mother cells, the short arm of chromosome 4 has a mean length of 22.47 μ compared with 36.31 μ for the long arm (LONGLEY 1939). It is not uniformly stained by aceto-carmine throughout its length but appears to have a more densely staining region around the centromere, which extends distally along the arms for a third or more of the distance to the ends. Nonhomologous pairing in the heterochromatic areas around the centromere makes it difficult to assign definite exchange points for translocations. It is possible also that such a differential staining reaction may be related to the amount of recombination between two gene loci or to the amount of suppression of recombination by the interchange in heterozygous translocations.

TRANSLOCATIONS USED AND PREVIOUS INFORMATION

Linkage data have been obtained for the 33 translocations listed serially in table 1. Certain of these have been used or referred to in various previous studies and

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[2] Journal paper No. 814 of the Purdue University Agricultural Experiment Station.

references to these are given in the last column of this table. The list of ANDERSON (1935) catalogs the known translocations to that time, and the papers by ANDERSON (1938, 1939) and ANDERSON and KRAMER (1954) summarize data on other chromosomes involved with 4 in interchanges. Chromosome 4 translocations have been used in such diverse studies as identifying chromosomes concerned with inheritance of such quantitative characters as smut resistance (BURNHAM and CARTLEDGE 1939; SABOE and HAYES 1941), locating centromeres on the linkage maps of maize (ANDERSON and RANDOLPH 1945), studying chromosome disjunction in interchanges with chromosome 6 (BURNHAM 1950) and selecting *su* kernels from segregating ears in early developmental stages for biochemical studies. (TEAS, CAMERON, and NEWTON 1952).

Those translocations for which no previous references are given are reported here for the first time. Their origins fall mainly into two series. The first series is derived from pollen irradiation of a *Wx Su Pr* stock by the late DR. L. J. STADLER who furnished seeds from pollinations made with the treated pollen. The second series stems from pollen irradiation of a *su wx pr* stock by DR. L. F. RANDOLPH with subsequent pollination of standard stocks carrying the normal alleles of these genes. Interchange lines were isolated from partially sterile plants grown from these seeds at the California Institute of Technology. Chromosomes involved in the interchange were identified by diakinesis tests following the method of ANDERSON (1935) or by linkage tests. Pachytene observations have since been made by LONGLEY.

PRESENTATION OF DATA

Cytological position and linkage with su

Cytological positions of the interchanges were determined from measurements made on three or four camera lucida drawings of pachytene chromosomes from plants heterozygous for the interchange. They are presented in columns two and three of table 1 as the average fraction of the distance from the centromere to the end of the arm involved. Thus, 4L.84 indicates a break far out on the long arm of 4 more than eight tenths of the distance from the centromere.

In columns four and five of table 1 are the recombination values for each interchange point with the *Susu* locus and the numbers on which these values are based. It will be noted that 19 values are below 5.0, 9 are between 5.0 and 12.0, and the remaining 5 are above 25.0. The cytological positions, however, indicate that the breaks are well distributed from 4S.79 to 4L.84. In the short arm the breaks appear to be uniformly distributed from about 4S.79 to 4S.09 but recombination values are low throughout. On the long arm, translocations in approximately the proximal third also show low recombination frequencies with *su*. Five translocations have breaks in the distal half of the long arm and these show from about 26 percent to almost 50 percent recombination. It appears, therefore, that in heterozygous translocations, suppression of recombination with *su* occurs when the interchange point is in the short arm or in the proximal third of the long arm. Further elucidation of this suppression effect is possible after considering the relative position of translocation points in the linkage map.

TABLE 1

A list of chromosome 4 translocations with cytological positions, linkage with su, *and references to previous literature*

Translocation	Position		Recombination with *su*		Reference*
	Chromosome 4	Other chromosome	No.	%	
T1-4a	4S.66	1L.49	832	3.5	A, 1935; S & H, 1941
T1-4c	4S.22	1L.33	614	2.4	
T1-4h	4L.65	1S.94	38	26.3	
T2-4a	4L.16	2L.29	361	3.3	A, 1935
T2-4b	4L.54	2L.88	215	48.8	A, 1935; S & H, 1941
T2-4c	4S.09	2L.77	1561	9.2	A, 1935; B & C, 1939; A & R, 1945
T2-4d	4L.50	2L.16	496	28.4	A, 1935; B & C, 1939
T2-4f	4L.13	2L.78	900	6.1	
T2-4g	4S.26	2L.13	997	2.7	
T2-4i	4S.37	2L.22	294	4.4	
T2-4j	4L.30	2S.19	322	1.2	
T2-4k	4L.18	2L.12	369	11.8	
T2-4l	4S.51	2L.56	345	5.8	
T2-4m	4S.46	2L.29	329	2.1	
T4-5b	4L.80	5L.70	165	43.0	
T4-5c	4S.45	5L.38	1667	1.1	B & C, 1939
T4-5d	4S.21	5L.19	385	3.4	B & C, 1939; A & R, 1945
T4-5j	4L.21	5L.40	247	5.7	
T4-5k	4S.13	5L.11	571	4.2	
T4-6a	4L.33	6L.44	1390	4.9	A, 1935; S & H, 1941; A & R, 1945; B, 1950; A, 1952
T4-6b	4S.79	6L.14	580	8.6	A, 1935; A & R, 1945; B, 1950
T4-6c	4S.29	6S.83†	707	3.7	A, 1935; A & R, 1945
T4-6e	4S.70	6L.60	592	4.9	
T4-7a	4S.27	7L.07	519	0.6	
T4-8a	4S.54	8L.23	1770	1.6	A, 1935; A, 1939
T4-8b	4S.12	8L.19	859	5.7	
T4-9a	4L.18	9L.50	1615	9.8	A, 1935; A, 1938; B & C, 1939
T4-9b	4L.84	9L.34	726	45.5	A, 1935; A, 1938
T4-9d	4L.11	9L.16	1326	3.8	
T4-9g	4S.31	9L.34	1348	3.3	
T4-10b	4L.16	10L.61	500	4.0	A, 1935; A & K, 1954
T4-10c	4S.12	10L.19	3420	1.1	A & K, 1954
T4-10e	4L.05	10L.03	783	3.3	A & K, 1954

*A = ANDERSON, A & K = ANDERSON and KRAMER, A & R = ANDERSON and RANDOLPH, B = BURNHAM, B & C = BURNHAM and CARTLEDGE, S & H = SABOE and HAYES, † = Interchange point in satellite.

Position of translocations in the linkage map

Four point backcross tests of 9 interchanges were made with *su*, *Tu*, and gl_3 and the data are presented in table 2. Additional three point tests of 5 of these and 14 additional interchanges were made with Ts_5 and *su*, or with *su* and *Tu*, and are summarized in tables 3–7. Tables 3 and 4 present tests with Ts_5 and *su* in which the breaks appear to be to the left and right of *su* respectively. Tables 5 and 6 summarize

TABLE 2

Four point backcross linkage tests with translocation points in chromosome 4

Translocation	Genotype of F_1	Parental combinations	Recombinations in region							Sum
			1	2	3	1-2	1-3	2-3	1-2-3	
T1-4a	+ *su* *Tu* gl_3	18	0	11	1	1	0	0	0	67
	T + + +	20	0	16	0	0	0	0	0	
T2-4a	+ *T* + +	53	1	9	26	1	0	1	0	187
	su + *Tu* gl_3	86	1	6	3	0	0	0	0	
T2-4b	+ + + *T*	27	16	2	5	0	4	0	0	99
	su *Tu* gl_3 +	19	18	1	5	1	0	0	1	
T2-4c	+ *T* + +	37	3	21	2	0	1	0	0	130
	su + *Tu* gl_3	38	2	18	7	0	0	1	0	
T2-4d	+ + *T* +	226	85	0	6	0	9	0	0	496
	su *Tu* + gl_3	112	45	1	11	0	1	0	0	
T4-5b	+ + + *T*	62	45	3	2	0	2	0	0	165
	su *Tu* gl_3 +	27	20	0	1	3	0	0	0	
T4-5d	+ *T* + +	35	1	11	9	0	0	1	0	103
	su + *Tu* gl_3	26	1	11	8	0	0	0	0	
T4-9a	+ *T* + +	8	4	5	5	0	1	2	0	99
	su + *Tu* gl_3	46	0	6	15	2	0	5	0	
T4-9b	+ + + *T*	132	77	14	35	4	23	0	2	556
	su *Tu* gl_3 +	123	58	21	40	5	19	1	2	

results with *su* and *Tu* which again differentiate translocations with breaks to the left of *su* from those in which the break appears to be between *su* and *Tu*. Table 7 presents data on *T2-4b* which confirms the results in table 1 showing the interchange distal to *Tu*. Additional two point tests with *su* have been included in the values presented in table 1. In all of these tables, the order of presentation of reciprocal crossover classes follows that of EMERSON, BEADLE, and FRASER (1935), and where different linkage phases were used they are indicated in the table headings by number and referred to in the body of the table.

Linkage tests in homozygous translocation stocks are frequently necessary to confirm the order particularly where suppression occurs in the heterozygous translocation. Results of such tests are summarized in table 8. Only tests with genes normally in chromosome 4, and those between chromosome 4 genes and genes in the other chromosome involved which serve to clarify the order in chromosome 4 are presented.

Summary of individual translocations

An attempt has been made in table 9 to summarize recombination values from tables 1 to 7 together with additional available 2 point data and to list the translocations in their approximate order from left to right on the chromosome. The follow-

TABLE 3

Backcross progenies from 1, +T+/Ts_5 + su; *and 2,* ++su/Ts_5T+

Translocation	Genotype	Parental combinations		Recombinations in region						Sum	Percent recombination	
				1		2		1, 2			Ts_5-T	T-su
T1-4a	1	239	167	0	0	12	6	0	0	424	0.0	4.2
T2-4g	1	127	101	8	5	0	9	5	0	255	7.1	5.5
T4-6b	1	147	136	2	0	13	19	2	1	320	1.6	10.9
T4-7a	1	98	61	10	7	0	1	1	0	178	10.1	1.1
T4-7a	2	44	24	6	3	0	0	0	0	77	11.7	0.0
T4-8a	1	—	181	—	3	—	9	—	0	193	1.6	4.7
T4-8a	1	167	139	3	3	0	0	1	0	313	2.2	0.3
T4-8a	2	55	60	4	4	0	0	1	0	124	7.3	0.8

TABLE 4

Backcross progenies from 1, ++T/Ts_5su+; *2,* +su+/Ts_5+T; *and 3,* +suT/Ts_5++

Translocation	Genotype	Parental combinations		Recombinations in region						Sum	Percent recombination	
				1		2		1, 2			Ts_5-su	su-T
T2-4c	1	303	212	62	59	30	28	10	6	710	19.3	10.4
T4-5c	1	252	213	28	18	0	0	0	0	511	9.0	0.0
T4-5c	2	57	53	9	7	0	1	0	4	131	15.3	3.8
T4-5d	1	102	77	13	12	0	0	2	2	208	13.9	1.9
T4-6a	3	171	177	32	25	7	10	4	3	429	14.9	5.6
T4-8b	1	326	195	80	31	21	15	4	7	679	19.4	6.9
T4-9a	1	200	151	34	27	48	47	6	3	516	13.6	20.2
T4-9b	1	25	55	4	4	34	39	4	5	170	10.0	48.2
T4-9d	1	28	37	6	5	0	0	0	0	76	14.4	0.0
T4-9d	2	403	303	144	77	15	21	4	0	967	23.3	4.1
T4-9g	2	50	37	7	4	2	2	1	0	103	11.7	4.9
T4-10b	1	113	120	20	11	3	1	6	5	279	15.1	5.4
T4-10c	1	83	69	20	1	0	0	0	2	175	13.1	1.1

ing brief description of each translocation will serve to indicate the basis for placing each translocation in its relative position in table 9. The tables in which data for each heterozygous translocation may be found are indicated and frequent reference to the data in table 8 for the translocation homozygotes is made.

T1-4a, tables 2, 3, 5 and 8. The translocation appears to lie near *su* and probably to the left since no recombinants with Ts_5 were found. The homozygous translocation tests were inconclusive in that 42.3 percent recombination between *su* and *Tu* does not differ significantly from independence for 123 individuals.

T2-4a, tables 2, 6 and 8. The translocation is between the centromere and *Tu* on the long arm. Homozygous translocation tests showed independence for *su-Tu.* The break in 2 is in the long arm and *Tu* shows linkage with *B* on the short arm of 2 while *su* does not.

T2-4b, tables 2, 7 and 8. The break is distal to gl_2; *su* and gl_2 are linked in the homozygote.

TABLE 5

Backcross progenies from 1, +suTu/T++; *and 2,* +su+/T+Tu

Translocation	Genotype	Parental combinations	Recombinations in region						Sum	Percent recombination	
			1		2		1, 2			*T-su*	*su-Tu*
T1-4a	1	68 72	0	0	25	30	0	3	198	1.5	29.3
T2-4g	1	41 29	1	0	9	15	4	0	99	5.1	28.3
T2-4i	1	72 43	1	5	39	19	0	3	182	4.9	33.5
T4-6b	1	57 31	4	4	18	24	2	0	140	7.1	31.4
T4-6b	2	27 26	1	0	7	6	2	2	71	7.0	23.9
T4-7a	1	12 16	0	0	8	5	1	0	42	2.4	33.3
T4-8a	1	86 60	1	0	20	23	0	0	190	0.5	22.6

TABLE 6

Backcross progenies from 1, +T+/su+Tu; *2,* +++/suTTu; *and 3,* ++Tu/suT+

Translocation	Genotype	Parental combinations	Recombinations in region						Sum	Percent recombination	
			1		2		1, 2			*su-T*	*T-Tu*
T2-4a	2	68 69	2	2	20	8	2	3	174	5.2	19.0
T2-4f	1	151 144	7	3	34	38	0	1	378	2.9	19.3
T4-5c	1	143 155	6	0	58	50	0	1	413	1.7	19.1
T4-5d	1	12 42	5	0	5	8	2	0	74	9.5	20.3
T4-6a	3	106 74	7	0	17	15	0	0	219	3.2	14.6
T4-6c	1	156 144	11	2	61	75	3	3	455	4.2	31.2
T4-9a	1	185 135	1	2	31	12	2	1	369	1.6	12.5
T4-9d	1	119 98	1	5	28	28	2	2	283	3.5	21.2
T4-9g	1	170 221	7	4	47	63	4	0	516	2.9	22.1
T4-10c	1	328 382	2	3	117	117	1	1	951	0.7	24.8

TABLE 7

Backcross progeny of T2-4b *from* ++T/suTu+

Translocation	Parental combinations	Recombinations in region						Sum	Percent recombination	
		1		2		1, 2			*su-Tu*	*Tu-T*
T2-4b	34 21	15	28	10	4	2	2	116	40.5	15.5

T2-4c, tables 2, 4, and 8. This translocation is between *su* and *Tu*. In the homozygote, Ts_5 and *su* remain linked but *su* and *Tu* do not. On chromosome two the break is well out on the long arm. Linkage of *su* with v_4 but not with *Ch* on 2 and linkage of *Tu* with *Ch* in the homozygote show that the break on 4 is in the short arm between *su* and the centromere and on the long arm of 2 proximal to *Ch*, confirming the cytological placement.

T2-4d, tables 2 and 8. The break on 4 is very close to *Tu* which is confirmed by the homozygous tests.

T2-4f, tables 6 and 8. The break is between *su* and *Tu*. The cytological evidence

TABLE 8

Summary of gene recombination in backcross tests of homozygous translocation stocks

Translocation	Region	Number of individuals	Recombinations	
			Number	Percent
T1-4a	*su-Tu*	123	52	42.3
	su-gl$_3$	316	147	46.5
T2-4a	*su-Tu*	882	444	50.3
	su-B	1399	721	51.5
	Tu-B	1568	690	44.0**
T2-4b	*su-gl*$_3$	1434	619	43.2**
T2-4c	*Ts*$_5$*-su*	504	63	12.5**
	su-Tu	579	293	50.6
	su-Ch	871	431	49.5
	Tu-Ch	429	193	45.0*
	su-v$_4$	1057	401	37.9**
T2-4d	*su-gl*$_3$	235	115	48.9
	su-B	235	107	45.5
	gl$_3$*-B*	235	68	28.9**
T2-4f	*Ts*$_5$*-su*	314	50	15.9**
	su-Tu	788	414	52.5
T4-5b	*su-Tu*	373	128	34.3**
T4-5c	*Ts*$_5$*-bm*$_1$	264	14	5.3**
	su-bm$_1$	394	13	3.3**
	su-pr	394	192	48.7
	Tu-pr	584	169	28.9**
T4-5d	*Ts*$_5$*-su*	495	100	20.2**
	Ts$_5$*-a*$_2$	281	83	29.5**
	su-a$_2$	460	103	22.4**
T4-6a	*Ts*$_5$*-su*	289	36	12.5**
	su-Tu	470	236	50.2
	Ts$_5$*-pl*	289	79	27.3**
	su-Pl	1158	226	19.5**
	Tu-Pl	470	228	48.5
T4-8a	*su-Tu*	331	150	45.3
	su-ms$_8$	750	316	42.1*
	su-j	1339	557	41.6**
T4-9a	*Ts*$_5$*-wx*	262	131	50.0
	Tu-wx	491	219	44.6*
T4-10b	*Ts*$_5$*-su*	314	111	35.4**
	Ts$_5$*-g*	143	47	32.9**
	su-g	157	13	8.3**

*P < .05 **P < .01.

indicates the long arm which is supported by the independence of *su* and v_4 in the homozygous translocation.

T2-4g, tables 3 and 5. The interchange is near *su*, but the order is uncertain.

T2-4i, table 5. Near *su*, order uncertain.

T4-5b, tables 2 and 8. At the position 4L.80 the interchange is definitely distal to *gl*$_3$.

T4-5c, tables 4, 6, and 8. Data from the heterozygous interchange indicate that

TABLE 9

The approximate order from left to right of chromosome 4 translocations

Translocation	Position in 4	Recombination					
		T No.	*su* %	*Ts*$_5$ No.	*su* %	*su-Tu*	
						No.	%
Probably distal to *Ts*$_5$							
*T4-6b**	S.79	580	8.6	320	10.6	260	29.6
Probably between *Ts*$_5$ and *su*							
*T1-4a**	S.66	832	3.5	424	4.2	265	32.4
*T2-4i**	S.37	294	4.4	—	—	182	33.5
*T2-4g**	S.26	997	2.7	255	8.6	99	28.3
*T4-8a**	S.54	1770	1.6	630	4.1	190	22.6
*T4-7a**	S.27	519	0.6	255	10.6	42	33.3
*T4-10c**	S.12	3420	1.1	175	13.1	951	25.1
Between *su* and centromere							
T4-5c	S.45	1667	1.1	642	10.3	413	27.6
T4-9g	S.31	1348	3.3	103	11.6	516	23.4
T4-5d	S.21	385	3.4	208	13.9	177	24.3
*T4-6c**	S.29	707	3.7	—	—	455	32.7
T4-8b	S.12	859	5.7	679	18.0	—	—
T2-4c	S.09	1561	9.2	710	19.3	130	35.4
Between centromere and *Tu*							
T2-4a	L.16	361	3.3	—	—	361	13.9
T4-9d	L.11	1326	3.8	1043	22.6	283	21.9
T4-10b	L.16	500	4.0	279	15.1	—	—
T2-4f	L.13	900	6.1	—	—	378	21.7
T4-6a	L.33	1390	4.9	429	14.9	219	17.8
T4-9a	L.18	1615	9.8	516	13.6	468	14.7
Distal to *Tu*							
*T2-4d**	L.50	496	28.4	—	—	496	28.2
Distal to *gl*$_3$							
T4-5b	L.80	165	43.0	—	—	165	42.4
T2-4b	L.54	215	48.8	—	—	215	40.5
T4-9b	L.84	726	45.5	170	10.0	556	34.2

* Order not established with certainty.

the break is near *su* but the order is not certain. Data from the homozygote testing the *Ts*$_5$-*su* interval would be desirable but are not available. The position, however, may be definitely established in the homozygous translocation by linkage tests of *Ts*$_5$ and *su* with *bm*$_1$ on the short arm of 5 and *su* and *Tu* with *pr* on the

long arm of 5. Both *Ts*$_5$ and *su* are closely linked with *bm*$_1$; *Tu* but not *su* is linked with *pr*. The break is therefore between *su* and the centromere on the short arm of 4 and between the centromere and *pr* on the long arm of 5 confirming the cytological placement.

T4-5d, tables 2, 4, 6 and 8. The translocation is between *su* and the centromere on the short arm of 4.

T4-6a, tables 4, 6 and 8. ANDERSON (1952) has completely characterized this interchange but chromosome 4 data are included here for completeness. The break is between the centromere and *Tu* on the long arm of 4.

T4-6b, tables 3 and 5. The interchange is to the left of *su* and near *Ts*$_5$. The order with respect to *Ts*$_5$ is uncertain but possibly to the left. The cytological position at 4S.79 is farther out on the short arm than any other chromosome 4 translocation.

T4-6c, table 6. Cytological evidence places the break at 4S.29 and the genetic data place it tentatively to the right of *su*.

T4-7a, tables 3 and 5. The translocation is very close to *su* but the order is not certain and must await data on the homozygous translocation.

T4-8a, tables 3, 5, and 8. Data from the heterozygous translocation show the break to be near *su* but do not permit the order to be definitely established; nor is the recombination between *su* and *Tu* conclusive in the homozygote. In chromosome 8, ANDERSON (1939) has given the order *T*-34.2-*ms*$_8$-10.9-*j* with the break in the long arm. A three point test with the translocation homozygote gave the order *su*-42.1-*ms*$_8$-6.7-*j* for 750 plants. Additional data for the *su-j* region in the homozygote gave 41.6 percent recombination for 1339 plants. Although the linkage of *su* with *ms*$_8$ and *j* favor a break position distal to *su*, linkage of *su* with *Tu* has not been demonstrated and the order remains uncertain.

T4-8b, table 4. The order appears definitely to be *Ts*$_5$-*su*-*T* and the cytological evidence places the break at 4S.12.

T4-9a, tables 2, 4, 6 and 8. The point of interchange is between the centromere and *Tu*. Since the long arm of 9 is also involved (table 1), *Tu* is linked with *wx* in the short arm of 9 while *Ts*$_5$ is not.

T4-9b, tables 2 and 4. This is probably the rightmost of all the translocations reported. Both the position at 4L.84 and the linkage show it to be well beyond *gl*$_3$.

T4-9d, tables 4 and 6. The order is definitely *Ts*$_5$-*su*-*T*-*Tu*. Cytological examination places the break on the long arm.

T4-9g, tables 4 and 6. Data in these two tables agree in placing the translocation to the right of *su* and the position is at 4S.31.

T4-10b, tables 4 and 8. Data on the heterozygous translocation indicate only that the break is nearer *su* than *Ts*$_5$. In the homozygote, however, *Ts*$_5$ and *su* remain linked, although the recombination value 35.4 is higher than normally expected. ANDERSON and KRAMER (1954) have indicated the order *T*-*g*-*R* on chromosome 10 with the interchange in the long arm of 10. In the homozygote, both *Ts*$_5$ and *su* are linked with *g* showing that they have remained with the centromere. The break in 4 is therefore on the long arm confirming the cytological position.

T4-10c, tables 4 and 6. The interchange is very near *su* but the order cannot be established with certainty.

The nature of recombination suppression

When the translocations are arranged in their approximate linear order and grouped by region, they show a definite pattern in the suppression of recombination in chromosome 4. Such an arrangement is presented in table 9 as are the *T-su*, *Ts*$_5$-*su*, and *su-Tu* recombination percentages.

The translocations with breaks in the short arm show little or no effect on *su-Tu* recombination. Seven translocations probably to the left of *su* give 29.2 percent *su-Tu* recombination while 5 to the right of *su* give 28.7 percent. Both averages compare favorably with the standard value of 29 given by EMERSON *et al.* (1935). Similarly, breaks in the long arm appear not to affect *Ts*$_5$-*su* recombination (average of 5 translocations 13.4 percent compared to the standard value of 15. The gradation from 22.6 percent for the break nearest the centromere to 10.0 percent at the most distal point is interesting but its significance if any is not apparent.)

More important is the effect in the arm in which the break occurs. In the short arm, *T4-6b*, the only translocation which appears to be distal to *Ts*$_5$, gave 10.6 percent recombination in the *Ts*$_5$-*su* region. Five translocations which most probably lie within the *Ts*$_5$-*su* interval gave values ranging from 4.1 percent to 13.1 percent with an average of 8.1 compared with the standard value of 15. In each case there is some uncertainty regarding the order and it is possible that the higher values belong to interchanges proximal to *su*. The group of 5 translocations with breaks proximal to *su* averaged 14.6 percent recombination for *Ts*$_5$-*su*. The gradation from 10.3 percent to 19.3 percent with the higher values for breaks near the centromere may be significant.

Similarly in the long arm, 5 translocations with breaks between the centromere and *Tu* gave *su-Tu* recombinations between 13.9 and 21.9 percent and averaged 17.8 as compared with a standard value of 29. Four translocations with breaks near or distal to *Tu* gave values from 28.2 to 42.4 with 3 values significantly above the standard value.

In interpreting the nature and distribution of crossing over suppression in chromosome 4, account also must be taken of the conspicuously low recombination between *su* and the break for all translocations except those in the distal half of the long arm, i.e. near *Tu* or beyond. In general, translocations tend to reduce crossing over in the immediate vicinity of the break and to have little effect on other regions. In accord with this general observation, the chromosome 4 data would indicate that there must be a long region between *su* and the centromere in which little crossing over normally occurs. The pattern in the long arm may well be the usual one of rather low frequency near the centromere increasing in the distal half of the arm.

On this basis the observed reductions in recombination fall into the following pattern: the only translocation indicated as distal to *Ts*$_5$ shows only slight and perhaps not significant reduction in *Ts*$_5$-*su* recombination. Translocations between *Ts*$_5$ and *su* show a pronounced reduction in *Ts*$_5$-*su* recombinations. A slight reduction was recorded for translocations proximal to but near *su* while other translocations between *su* and the centromere show no reduction either in the *Ts*$_5$-*su* or the *su-Tu* region. Translocations in the long arm proximal to *Tu* show a distinct reduction in

su-Tu recombinants (29 to 17.8 percent) while the *su-T* values remain below 10 percent. The reduction thus appears to be in the more distal regions of the centromere-*Tu* region. Translocations beyond *Tu* show an abrupt rise to normal *su-Tu* values and more than 28 percent recombination between *su* and the translocation.

SUMMARY

Linkage relationships of 23 translocations involving chromosome 4 with the Ts_5, *su*, *Tu*, and gl_3 loci are reported. The recombination between the interchange point and *su* were obtained for an additional 10 translocations.

Of the 23 in which order could be determined, seven are probably to the left of *su*, six on the short arm between *su* and the centromere, six on the long arm between the centromere and *Tu*, one between *Tu* and gl_3, and three to the right of gl_3.

In heterozygous translocations, interchanges in the short arm between *su* and the centromere appear to have little effect in suppressing recombination between Ts_5 and *su*, or between *su* and *Tu* even though this region includes the *su*-centromere region. Interchanges in the centromere-*Tu* region, however, cause a definite decrease in *su-Tu* recombination. It is suggested that there is an appreciable region of undetermined length between the centromere and the *su* locus in which there is little recombination in normal stocks and that interchanges in this region therefore have little effect on recombination.

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THE USE OF LINEAR ASCI FOR CHROMOSOME MAPPING IN SACCHAROMYCES

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IN yeast, as in other organisms where tetrad analysis is feasible, it is possible to determine the distance between a given gene and the centromere of its chromosome. This may be accomplished directly if the relationship of the spores in the ascus is known. Two such relationships have been demonstrated. In the four-spored asci of *Schizosaccharomyces pombe*, the two spores at each end of the ascus are sisters (LEUPOLD 1950). An ascogenous cell of genotype *A a* produces an ascus in which spores of composition *A*, *A*, *a*, and *a* are arranged in that order if there has been no recombination between the locus of *A* and the centromere. If a crossover has occurred in this region, the following sequences, indicative of second-division segregation, are obtained: (*A a a A*), (*a A A a*), and (*A a A a*). In *Saccharomycodes ludwigii*, the distribution of the nuclei is such that the two spores at each end of the ascus are non-sisters (WINGE and LAUSTSEN 1939). If the nuclear distribution is random otherwise, the following noncrossover spore arrays would be expected: (*A a a A*), (*a A A a*), and (*A a A a*). If recombination has occurred between *A* and the centromere, the same three arrays would be expected half the time, and a fourth array (*A A a a*) the other half. An estimate of the frequency of second-division segregation for a gene in this organism would therefore be twice the frequency of (*A A a a*) asci. The consequence of a non-random nuclear distribution in *S. ludwigii* will be discussed later.

In linkage studies in Saccharomyces hybrids, it has been customary to dissect asci of the oval type, in which spore relationship is not known. Segregation data obtained from these asci have been used in chromosome mapping by LINDEGREN (1949a, 1951). The equations for calculating gene-centromere linkage in unordered tetrads have been given by PERKINS (1949) and WHITEHOUSE (1950). This method is, however, indirect; in order to determine whether or not a given gene exhibits linkage with the centromere, it is necessary to have segregating in the same asci another gene which exhibits centromere linkage and for which the recombination frequency is known. Saccharomyces hybrids also produce asci which are elongated and in which the spores are arranged in a linear series. Hybrids may be selected which yield as many as 40 percent of linear asci. The investigation reported below was undertaken to determine the pattern of spore array in these asci, for the purpose of ascertaining their usefulness in gene-centromere linkage studies.

TYPES OF SEGREGATIONS EXPECTED IN LINEAR ASCI

When this study of the linear asci of Saccharomyces hybrids was begun, the consequences of three types of nuclear distribution in the ascus after meiosis were considered. They were: (1) the distribution by spindles in a line as in *Schizosaccharomyces*

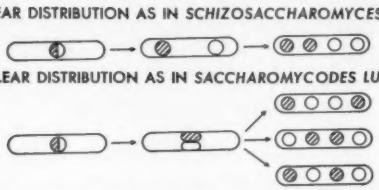

FIGURE 1.—The possible orientations of nuclei in linear asci. The types of asci given for *S. ludwigii* are those expected if the nuclei at each end are non-sisters and are otherwise arranged at random.

pombe, (2) the distribution by parallel spindles as in *Saccharomycodes ludwigii*, and (3) a random distribution resulting from a slippage of the nuclei in the elongated ascogenous cell. The first two distributions, which result in a definite orientation of the nuclei in the linear ascus, are diagrammed in figure 1. A distinction between the three types of nuclear distribution during meiosis is possible by the use of the linear array. As MATHER (1935) has shown, the maximum percentage of second-division segregation, if there is no interference, is 66.7 percent, which is the limit attained with an infinite number of exchanges between the locus and the centromere. This means that if the nuclear distribution is of the *S. pombe* type, for a given gene the frequency of asci with the noncrossover spore array, (*A A a a*), will have a lower limit of 33.3 percent. If the nuclear distribution is by means of parallel spindles, the frequency of asci with the crossover array (*A A a a*) will have an upper limit of 33.3 percent. If there is a random distribution of the nuclei, the spore array (*A A a a*) will be expected for every gene, linked or unlinked with the centromere, in a third of the asci. For a gene showing no centromere linkage, the four spore arrays (*A A a a*), (*A a a A*), (*a A A a*), and (*A a A a*) will be expected in a ratio of 2:1:1:2, regardless of the type of nuclear distribution.

MATERIALS AND METHODS

Preliminary crosses were made to incorporate as many characters as were available into a single hybrid. The hybrid obtained was heterozygous for the genes controlling seven characters that were readily scored and which gave 2:2 segregations in nearly every ascus. These characters were mating type, galactose and melibiose fermentation, and histidine, tryptophane, uracil, and methionine independence.

The yeast stocks were maintained on a nutrient agar containing 1% peptone, 1% yeast extract, and 2% dextrose. For the mating type tests a broth medium of the same composition was used; the segregants were classified as *a* or α as a result of crosses with strains of known mating type. The galactose fermentation tests were made in Durham tubes using a broth of 1% peptone, 1% yeast extract, and 2% galactose. The tests for the fermentation of melibiose were made in Kahn tubes containing 1½ cc of the following medium adjusted to pH 6.0: 1% peptone, 1% yeast extract, 2% melibiose, and the indicator bromcresol-purple. The nutritional requirements were scored in media with a basal composition of Bacto-Vitamin Free Yeast Base, less histidine, tryptophane, and methionine, and with the addition of biotin, pyridoxine hydrochloride, and thiamine hydrochloride, a formula of WICKERHAM's (1953).

Three clones of the hybrid were dissected. Two of these, No. 1002 and No. 1004, were obtained by isolating with the micromanipulator single diploid cells from a mass mating of the haploid parents. The third diploid, No. 1003, was obtained by following the procedure suggested by POMPER AND BURKHOLDER (1949), i.e. by plating the mating mixture on a minimal synthetic medium which lacked the above four essential nutrients and then selecting one of the prototrophic clones.

Linear asci in which the ascus envelopes were slightly longer than that needed to confine the four spores were selected for dissection; a group of suitable asci is pictured in plate I-F. The order of the spores in the ascus was retained by cutting the tip of the ascus and then squeezing out the spores one at a time for transfer to droplets of nutrient medium. If, during a dissection, a spore was crushed or the order of the spores was lost, the ascus was abandoned.

RESULTS

From about 150 dissections of the hybrid resulting from the cross of haploid parents of these genotypes, (*a g ME HI tr MT ur*) and (*α G me hi TR mt UR*), 74 asci were obtained from which all four spores germinated and produced colonies that could be transferred and tested. In the 74 asci, the characters mating type, galactose and melibiose fermentation, histidine, tryptophane, and methionine independence gave the expected 2:2 ratios in every instance. For uracil independence, there were three asci with irregular ratios and these were not included in the analysis of the segregations for this character.

In order to detect linkage and to apply the equations of WHITEHOUSE (1950) to determine centromere linkage in unordered tetrads, the characters were considered in pairs. When the segregations for the genes two at a time are studied, there are three categories for the asci based on the genotypes of the spores. Following the terminology of PERKINS (1953), a diploid hybrid of genotype (*A B /a b*) will yield parental ditype asci (*AB AB ab ab*), non-parental ditype asci (*Ab Ab aB aB*), and tetratype asci (*AB Ab aB ab*). In table 1 the possible pairings of the seven characters are scored as to the three types of asci and in addition the proportion of the tetratype asci is calculated. The data in table 1 indicate that there is no close linkage between any of the genes since there is no preponderance of the parental ditype asci for any of the combinations. From the consideration of the proportion of tetratype asci, it is also apparent that no two of the genes are closely linked to the centromere, since centromere linkage for a pair of genes would be indicated by a proportion of tetratype asci significantly less than two thirds. In every instance, the fraction is so nearly two thirds that it is impracticable to use the equations for the determination of centromere linkage in unordered tetrads.

When the criterion of spore array was applied to the same data, evidence of close linkage with the centromere was obtained for the tryptophane locus. The asci were scored as to spore array, (*A A a a*), (*A a a A*), (*a A A a*), or (*A a A a*), for each character. In the segregations for tryptophane independence, 70 of 74 asci were of the fourth array (table 2). This was interpreted as evidence that the fourth array, (*A a A a*), is the noncrossover class and that the gene *TR* is closely linked to the centromere of its chromosome. This would mean that the distribution of the nuclei during meiosis is as diagrammed in the last series for *S. ludwigii* in figure 1, with

TABLE 1

Tetrad analysis of the diploid hybrids, 1002, 1003, and 1004 to determine gene-to-gene and gene-to-centromere linkage

Gene pair	Ditype asci		Tetratype asci	Proportion tetratype
	Parental	Non-parent		
Tryptophane-Mating type	22	10	42	.57
Tryptophane-Galactose	15	11	48	.65
Tryptophane-Melibiose	9	15	50	.68
Tryptophane-Histidine	9	8	57	.77
Tryptophane-Uracil	13	13	45	.63
Tryptophane-Methionine	12	11	51	.69
Mating type-Galactose	14	12	48	.65
Mating type-Melibiose	11	16	47	.64
Mating type-Histidine	12	15	47	.64
Mating type-Uracil	10	18	43	.61
Mating type-Methionine	8	13	53	.72
Galactose-Melibiose	15	6	53	.72
Galactose-Histidine	12	13	49	.66
Galactose-Uracil	10	6	55	.78
Galactose-Methionine	11	10	53	.72
Melibiose-Histidine	14	8	52	.70
Melibiose-Uracil	14	12	45	.63
Melibiose-Methionine	19	13	42	.57
Histidine-Uracil	12	7	52	.73
Histidine-Methionine	8	20	46	.62
Uracil-Methionine	8	19	44	.62

TABLE 2

The segregations of the diploid hybrids, 1002, 1003, and 1004 classified according to type of array found in the ascus

Locus	Spore arrays				Percent array IV (noncrossovers)
	I + + − −	II + − − +	III − + + −	IV + − + −	
Tryptophane	1	1	2	70	95
Mating type	26	6	11	31	42
Galactose	20	16	14	24	32
Melibiose	25	18	9	22	30
Histidine	19	16	23	16	22
Uracil	24	11	11	25	35
Methionine	29	13	10	22	30

non-sister nuclei alternating in the ascogenous cell. A single crossover would give an ascus with one of these three arrays, (*A A a a*), (*A a a A*), or (*a A A a*), which are the second division segregation classes. Since the noncrossover class is limited to a single spore array, (*A a A a*), these asci can be conveniently used to determine centromere linkage. For the loci other than the tryptophane locus, there was no evidence of centromere linkage.

On the above assumptions, the gene *TR* can be placed 2.5 units from the centromere since about five percent of the asci indicated second-division segregation. Confirmatory evidence of this close centromere linkage was obtained in tetraploid material, in which linkage with the centromere can be determined for individual loci (ROMAN, PHILLIPS, and SANDS 1955).

The use of tetraploid hybrids to determine centromere linkage for the gene controlling tryptophane independence

A tetraploid hybrid of the genotype, *A A a a*, will produce asci which show 4:0, 3:1, and 2:2 segregations for the phenotype of the dominant allele. The frequency of these segregations may be predicted for a character when the percentage of second-division segregation is known. For a gene which shows five percent second-division segregation in the diploid hybrid, the following percentages for the three types of asci from the tetraploid will be expected if there is bivalent pairing of the homologous chromosomes during meiosis: 4:0 asci 63.3 percent, 3:1 asci 6.7 percent, and 2:2 asci 30 percent. If there is tetravalent pairing, the following percentages will be expected: 4:0 asci 63.5 percent, 3:1 asci 5.4 percent, and 2:2 asci 31.1 percent.

For the 48 asci of the tetraploid hybrid No. 1076, which was of the genotype *TR TR tr tr*, the following segregations were obtained: 28 (4:0) asci, 2 (3:1) asci, and 18 (2:2) asci. The expected numbers on the basis of bivalent pairing and a second-division segregation frequency of five percent are 30.2 (4:0) asci, 3.4 (3:1) asci, and 14.4 (2:2) asci. If pairing is tetravalent, the expected numbers are nearly the same, i.e., 30.5 (4:0) asci, 2.6 (3:1) asci, and 14.9 (2:2) asci. A chi-square analysis shows that the observed frequencies of the segregations for tryptophane independence are in good agreement with the frequencies expected if *TR* is 2.5 units from the centromere, as the evidence based on the linear array indicates.

Cytological evidence on the distribution of the nuclei during meiosis in Saccharomyces hybrids

A tetraploid clone, No. 1F5, was chosen for a cytological study; since upon sporulation, 30 to 40 percent of the asci were linear and the cells were about half again as large as the average diploid cell. To induce sporulation the procedure suggested by LINDEGREN (1949b) was followed. Cells from a two days growth on presporulation medium were transferred to a gypsum slant. After 20 to 24 hours on the slant, the ascogenous cells were placed on cover slips and fixed in osmic acid vapors and Schaudinn's fluid, following the procedure ROBINOW (1944) recommended for nuclear stains of bacteria. The fixed cells were then treated with ribonuclease for about an hour and a quarter at 55°C, and then stained for a half hour with Giemsa stain. Some of the cells that were photographed were mounted in phosphate buffer. It was found however, that in many cases a quick passage through alcohol solutions and mounting in "Euparal" greatly intensified the staining of the nuclei.

No meiotic figures could be discerned although the nuclei were comparatively large as can be seen in plate I-A, which shows a typical elongated ascogenous cell with a single nucleus. When two nuclei were seen, they were always found together near the center of the elongated ascogenous cell in the position indicated in plate I-B. In many

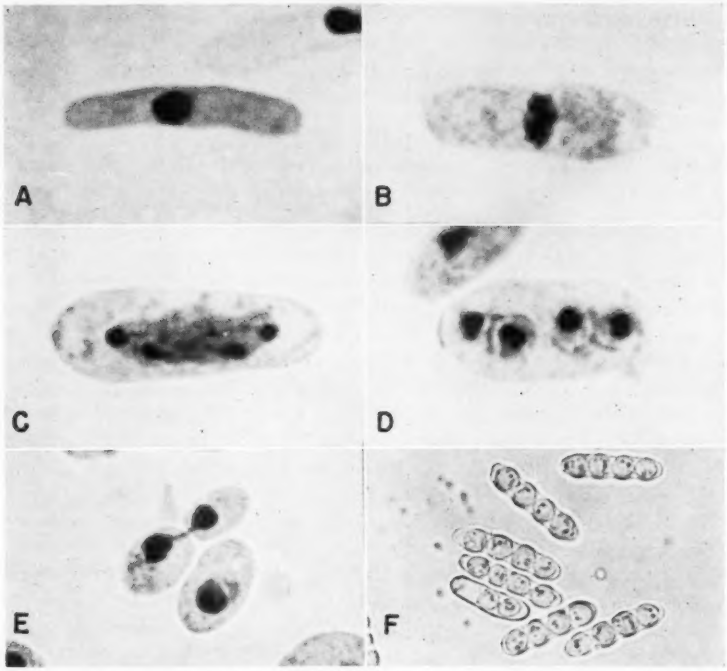

PLATE I.—A-D, ascogenous cells of a Saccharomyces hybrid, Giemsa stain. E, vegetative cells, Giemsa stain. F, linear asci, unstained.

cells in which four nuclei were seen, a thin strand of chromatinic material connecting the sister nuclei was observed, so there was no doubt as to the relationship of the nuclei. An example of this is seen in plate I-C, which shows the alternation of the non-sister nuclei in the ascogenous cell. The final alignment of the four nuclei in the elongated ascogenous cell, just prior to the formation of the spores, is seen in plate I-D. In a thorough inspection of many preparations of this hybrid, No. 1F5, and of other tetraploid and diploid Saccharomyces hybrids from our stocks, stained with Giemsa and by the Feulgen technique, there never were seen any nuclear configurations that would indicate a nuclear distribution like that found in *Schizosaccharomyces pombe*.

The elongated ascogenous cells were found only in material which had been induced to sporulate. The vegetative cells were typically oval and quite regular. In preparations of vegetative cells that were made to illustrate this, no elongated or multinucleated cells were found. A typical mother cell with a bud and a strand of chromatinic material connecting the nuclei of the two is seen in plate I-E.

DISCUSSION

The results from the scoring of tryptophane independence in 74 linear four-spored asci of a diploid Saccharomyces hybrid indicated that the nuclear distribution during

meiosis resulted in the alternation of non-sister nuclei in the elongated ascogenous cell. This manner of distribution is similar to and may be the same as the nuclear distribution in *Saccharomycodes ludwigii*; for in the description by WINGE AND LAUSTSEN (1939) it was proposed only that the nuclear distribution for this organism was by means of parallel spindles with no stipulation as to the final alignment of the nuclei other than that the two spores at one end of the ascus contained non-sister nuclei.

Of the seven genes that were heterozygous in the diploid hybrid, only one, *TR*, showed close centromere linkage. For one other gene, mating type, 58 percent of the asci were of the second-division segregation arrays. The deviation of the observed number of second-division segregation asci, 43, from the number expected for a gene showing no centromere linkage, 49, is not significant at a 95% level. However, when the data for mating type from all crosses where linear asci were dissected are summarized, the following figures are obtained: Array I, 141 asci; Array II, 68 asci; Array III, 88 asci; and Array IV, 261 asci. For the 558 asci, the observed number, 297 asci, showing second division segregation differs significantly from the 372 asci expected if this locus showed no centromere linkage. In this compilation, 53.3 percent of the asci show second-division segregation. LINDEGREN (1949a), using unordered tetrads, has mapped the locus of the mating type gene at 24 units from the centromere. If with the above data, the centromere linkage for the locus of mating type is taken as one half the percentage of second-division segregation, the value obtained, 27 units, is in reasonable agreement with LINDEGREN's figure.

It had been anticipated that the gene for galactose fermentation would show close centromere linkage since LINDEGREN (1949a) has this locus mapped at 6 units from the centromere. Since the galactose gene of this hybrid showed no linkage with the centromere, this was a point of complete disagreement until crosses made with a galactose-negative haploid, supplied through the courtesy of DR. LINDEGREN, showed that we were dealing with two different galactose genes. We have designated our locus as galactose-2. A summary of the data from 348 linear asci indicates 70.7 percent second-division segregation for galactose-2.

A possible source of error in the use of the spore array in centromere mapping would occur if there were an occasional failure of the non-sister nuclei to alternate in the ascus. Such an ascus would be improperly scored as to second-division segregations. The chance for an error of this type, however, is negligible, in this series of dissections at least, since, as indicated by the scoring for tryptophane independence, there would be at most only four possible deviations from the normal nuclear distribution in these 74 asci.

The extra care required to dissect out the spores in order from a linear ascus is justified since the spore arrays provide a direct measure of the degree of second-division segregation while only an estimate of this may be obtained by using the equations for determining centromere linkage in unordered tetrads.

SUMMARY

On the basis of genetic evidence, it was concluded that the nuclear distribution in the linear four-spored asci of a diploid Saccharomyces hybrid follows a definite pattern with the alternation of spores with non-sister nuclei in the ascus. A cytological

investigation of the nuclear distribution during meiosis also indicated that there was an alternation of non-sister nuclei in the elongated ascogenous cell. Thus, a deviation from the alternation of the dominant and recessive characters in the scoring of these asci indicates the occurrence of a crossover and gives a simple and direct means to determine the linkage of a gene to the centromere of its chromosome.

From the analysis of 74 linear asci of the diploid hybrid, the gene for tryptophane independence was mapped at about 2.5 units from the centromere, a value which was confirmed independently in tetraploid material. Evidence is also given that the mating type locus is approximately 27 units from the centromere. The genes for melibiose and galactose (*G-2*) fermentation, histidine, uracil, and methionine independence did not show centromere linkage.

ACKNOWLEDGMENTS

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PLUMAGE COLOR ALLELISM IN THE RED JUNGLE FOWL (GALLUS GALLUS) AND RELATED DOMESTIC FORMS

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THE lack of an ancestral "standard of reference", "normal" or "wild type" for use in genetic studies of the domestic fowl has been of little concern to many poultry geneticists (for a recent discussion of this topic see JAAP and HOLLANDER 1954). In the field of plumage color inheritance, however, a standard of reference seems necessary in describing the action of mutant genes. What the standard should be has yet to be determined, since the ancestry of the domestic fowl is still unknown. One or more of the four known species of jungle fowl may have contributed to the ancestry of the fowl, or some yet undiscovered fossil prototype may have been the major contributor. In view of this uncertainty, the use of the red jungle fowl (*Gallus gallus* L.) as a standard of reference for mutant genes in the fowl is entirely unwarranted without definite proof of allelism to genes manifest in the red jungle fowl. However, the lack of proof of allelism of genes to those of red jungle fowl need not disprove the "wild type" nature of a given allele since other jungle fowl species may have been involved.

Crosses between domestic breeds and red jungle fowl will be reported herein which demonstrate that a multiple allelic series of genes controls the differentiation of certain plumage colors and patterns. On this evidence, wild type designation of the character expressed in the red jungle fowl is justified.

Only a few attempts have been made to correlate the many color patterns in the down of chicks with adult plumage type. Often no correlation exists. In the multiple allelic series herein described, however, the same alleles are shown to regulate plumage color patterns in both the chick and the adult.

The three other groups of multiple alleles reported for the domestic fowl affect leg morphology (polydactylism, WARREN 1941; 1944), rate of feather growth (JONES and HUTT 1946), and blood groups (BRILES *et al.* 1950).

HISTORY

The phenotype represented in the brown Leghorn and red jungle fowl is commonly called black-red or black-breasted red and is based on the phenotype of the cock. KIMBALL (1951) was one of several workers who attempted to analyze the black-breasted red complex in the domestic fowl, and later (1952a, b) he published evidence of relationships of patterns in the down with respect to the wild type down pattern in the red jungle fowl and many domestic breeds. His analysis led him to assign the symbol e^+ to a gene governing wild type plumage and down. KIMBALL'S reason for assigning the symbol e^+ to the black-breasted red pattern was that he found the latter to be allelic to a dominant gene *E* (extended black) which produces self black in both sexes of adults and chicks. The genetic basis for extended black was first recognized

by LIPPINCOTT (1918). KIMBALL also assumed allelism to *e* (Columbian restriction) first described by DUNN (1923). This gene (*e*) restricts black pigment to the feathers of the neck, wings (primaries and secondaries) and tail. The relationship of *E* to *e*, however, is not always clear. COCK and PEASE (1951) state that in their work with the brussbar (barred black-red), F_1 heterozygotes (brussbar × Columbian) backcrossed to brussbar produced progeny in which ". . . the proportion of black-reds (or their silver counterparts) varies widely in different families, and in most is too low to be explained by a single gene." It is obvious from the above account that much more evidence is to be needed on the *extended black-Columbian restriction-black-red* relationship.

PEASE and COCK (1951) have extracted from the light Sussex breed of fowl a recessive gene called "retarded"* which, apparently, in homozygous state dilutes the down of chicks from a very pale striped wild type, with reduced mid-dorsal back stripe, to an all white chick. The adults (black-reds) are not similarly affected—cocks are more intensely colored; and hens are predominantly salmon-chestnut throughout with paler salmon breasts, black in tail and wing primaries, and the neck of deeper chestnut tint. PEASE and COCK also feel that the retarded gene may be found in Indian game (dark Cornish) fowl and in the salmon Faverolle breed. Regarding the latter breed, KIMBALL (1952b) states that the non-striped white down of the salmon Faverolle (and wheaten game) is governed by a dominant dilution gene, *Wh* (wheaten). Heterozygotes (*Whwh*), however, have white down with a mid-dorsal chestnut band and no black stripes. It seems evident that the retarded gene of PEASE and COCK, apparently completely recessive, may not be the same gene *Wh* which KIMBALL has described. Furthermore, KIMBALL (1953b) states that homozygous *WhWh* (wheaten game) cocks are indistinguishable from black-red (wild type) cocks, except for white undercolor of plumage; hens (*WhWh*) are of a red wheat coloration with no stippling, and black pigment is found in rectrices and remiges.

The author (1953) described a recessive mutation (yellowish-white down) discovered in a red jungle fowl cock of Siamese origin. The effects of this gene were to dilute the down of chicks of both sexes to a yellowish-white (fig. 1, D) and to depigment or dilute the plumage of juvenile and adult hens to creamy-buff or cinnamon. Adult cocks were not affected and were indistinguishable from normal wild type cocks. The gene was completely recessive, and in adult cocks homozygous for the gene, the undercolor of the plumage was not affected.

It can be seen from the above that three very similar phenotypes prevail: wheaten, retarded and yellowish-white. Minor differences in them can be accounted for in several ways. The two most logical are, (1) different genetic backgrounds (polygenes) affecting the expression of a mutant gene, or (2) three non-allelic genes producing similar phenotypic effects (a phenomenon not infrequently found in other animal forms). Genetic differences, if any, will only be found when the three phenotypes are brought together and analyzed genetically in proper matings.

* The term "retarded" has been used by WARREN (1933) for a condition affecting rate of growth of feathers. The symbol l^a has been given to this gene by JONES and HUTT (1946). The term is used here only with reference to the phenotype described by PEASE and COCK (1951).

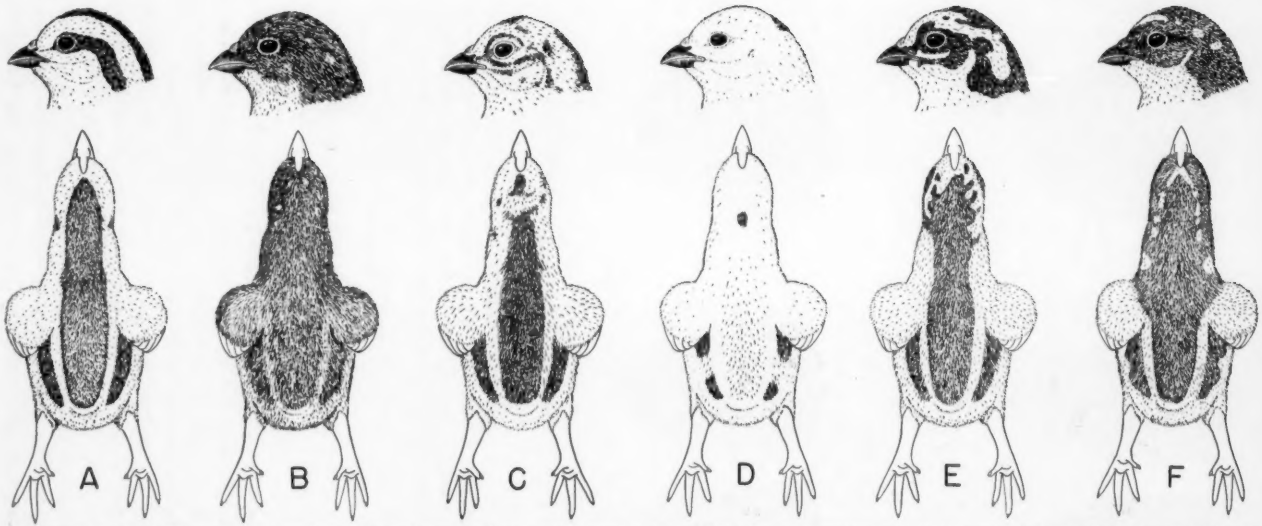

FIGURE 1.—Semi-diagrammatic drawings of lateral views of heads and dorsal views of chick down phenotypes: A, wild type striped (e^+e^+); B, brown (e^be^b); C, speckled head (e^se^s); D, yellowish-white (e^ye^y); E, heterozygous speckled head (e^be^y); and F, heterozygous brown head (e^be^s).

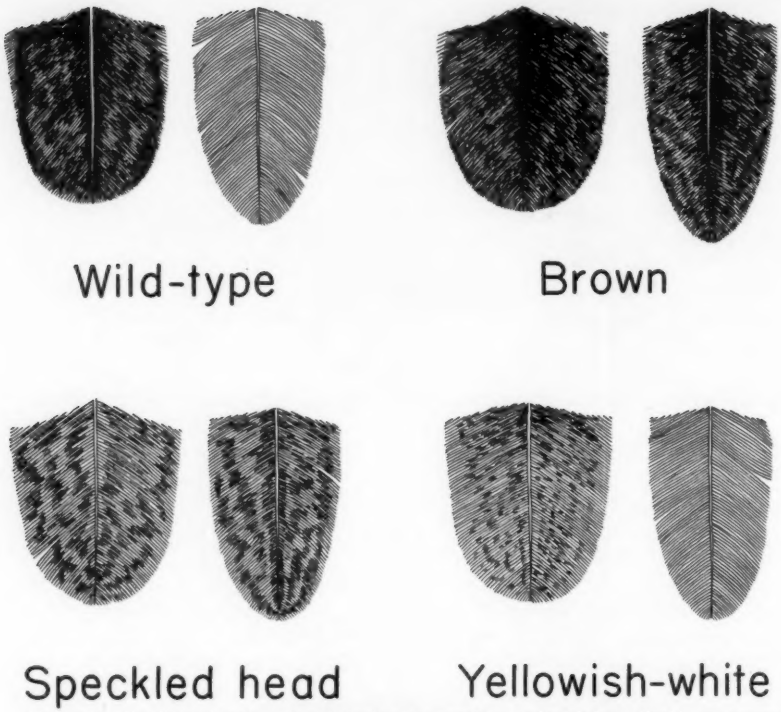

FIGURE 2.—Feather apices from the mid-back and upper breast of adult hens. Within each group the feathers from the back are at the left; feathers from the breast are at the right. Wild type (e^+), brown stippled feather of back and non-stippled salmon-colored feather from breast; brown (e^b), brown stippled feathers; speckled head (e^s), grayish-brown lightly stippled feathers; yellowish-white (e^y), cinnamon-buff lightly stippled feather of back and salmon-colored feather of breast.

DESCRIPTION

Since the primary concern of this investigation was with mutants affecting the wild type plumage and down pattern, the following description of the wild type, as seen in the red jungle fowl, is in order:

Cocks—Lanceolate orange-yellow feathers on the neck and rump; bay colored secondaries; blackish-brown primaries; and metallic-green tail feathers.

Hens—(fig. 2) Stippled (black spots on greyish-brown to brown ground color); light, salmon-colored breasts; and yellow-orange neck feathers.

Chicks—(fig. 1, A) The head has a median dorsal stripe, dark brown to light brown; it extends along the back of the head to a point along the neck. This stripe gradually disappears on the lower neck and continues wider along the dorsal surface of the back and rump. Lateral to this mid-dorsal back stripe there is a whitish-yellow stripe (approximately 5–8 mm in width) extending from a point lateral to the pygostyle and running anteriorly to a point along the scapula at the base of the wing. This

stripe is margined along the ventral edge with black. A line of dark brown down runs through the eye to a point posteriorly on the side of the head, slightly above and posterior to the auditory orifice. The other areas of the down on the chick vary from yellowish-white to buffy-yellow.

The phenotype of these chicks is presumed to be an expression of the gene e^+ which theoretically controls in part adult wild type phenotype. Probably several genes contribute to the production of a perfected wild type plumage color. In conformity with current usage, alleles of this locus will be designated with superscript letters. Use of the symbol e^+ is based on data which shows that other mutant alleles of e^+ are allelic to E (extended black). The symbol e^+ is herein used only with reference to the normal or wild type birds used in this study and does not necessarily assume identity with other genes. Mutants used in this study were obtained in several ways. The red jungle fowl mutants (yellowish-white) were originally obtained from matings involving a heterozygous Siamese cock obtained from a game bird breeder (see MOREJOHN 1953). A strain of dark Cornish purchased from a breeder of commercial cross-bred poultry was also used and found to carry the same mutant allele (yellowish-white). The new mutant alleles to be described were discovered originally in a brood of day-old brown Leghorn chicks obtained from a commercial poultry hatchery. Extended black birds originally derived from the Australorp breed of domestic fowl, were obtained from DR. E. M. PLOCHER of Watsonville, California.

Following is a list of the mutants to be discussed. All adult cocks were phenotypically wild type.

Brown, (e^b)

The phenotype of the chick (fig. 1, B) may be described as being primarily all brown. The back was darker than the underside. Occasional small light brown spots seemed to delimit what may be taken to be the outer edge of the dorsal head stripe as found in wild type chicks. Dorso-lateral light brown striping was greatly suppressed and in most cases was absent. The adult hen differed from the wild type hen in being dark brown stippled throughout and not having a salmon-colored breast (fig. 2). Shanks and toes very frequently showed dark bluish-brown pigment in individual scales, and brown pigment was distributed along the distal edges of most scales.

Speckled head, (e^s)

The phenotype of the chick (fig. 1, C) was somewhat variable. In general, the body down color was very similar to the normal wild type striped phenotype. In the head region, however, the mid-dorsal head stripe was irregularly represented. Several spots and streaks also occurred in the area between the dorsal head stripe and the eye stripe and often were united giving the appearance of spectacles around the eyes. Adult hens (fig. 2) were similar to e^b homozygotes but were not as dark, i.e., feathers were not as darkly stippled.

Yellowish-white, (e^w)

The chick phenotype (fig. 1, D) appeared to be an extreme dilution of buff and brown pigment. The chick was uniformly yellowish-white with no head stripes and only a vague suggestion of the mid-dorsal brown stripe. The dark outer edges of the

mid-dorsal stripe and the dorso-lateral black stripes were variable. Adult hens (fig. 2) were creamy-buff in color with dark stippled feathers of the back and rump. Hackles of the neck were yellow-orange, and the breast was light salmon-colored. Red jungle fowl homozygous for e^y were used in some of the experiments to follow, and a strain of dark Cornish was used which was demonstrated to carry the allele e^y.

EXPERIMENTAL MATINGS

Three parallel groups of breeding experiments were undertaken involving the groups of alleles: (I) e^+, e^b, e^s, and e^y; (II) e^+, e^b, e^s; and (III) *E*, e^b, e^s. Group I matings employed brown Leghorns and red jungle fowl; group II matings consisted only of matings between brown Leghorns; and group III matings were between brown Leghorns and extended black birds derived from the Australorp breed of fowl. A series of matings (IV) between brown Leghorn, dark Cornish and red jungle fowl were also undertaken to investigate the possibility of linkage of e^+ with pea comb (*P*). In the following experiments it should be kept in mind that in all cases except with extended black, all cocks were phenotypically e^+ as adults, i.e., only hens showed the mutant phenotype. Both sexes were equally affected as chicks.

I. *Genetic tests with* e^+, e^b, e^s, *and* e^y. Crosses were made between brown Leghorn mutants and red jungle fowl mutants. In table 1, the results of matings (1), (2), (3), (4), (5), (6) demonstrate that e^y is allelic to both e^+ and e^b. The progeny of mating (7) is important in that it represents the only evidence that e^s and e^y are not at different loci since the wild type striped phenotype was not realized.

II. *Genetic tests with* e^+, e^b, *and* e^s. Brown Leghorns alone were used in these matings. Matings (1) and (2) of table 2 demonstrate homozygosity of mutants e^b and e^s. The relation of these two alleles to e^+ is clear in mating (3) producing a 2:1:1 ratio together with the 1:2:1 segregation of e^b and e^s homozygotes from e^be^s individuals of mating (4). Considered with matings (5), (6), (7), (8), and (9), these data support the concept of a series of alleles of e^+.

III. *Genetic tests with E,* e^b, *and* e^s. Heterozygous cocks and hens of the genotype

TABLE 1

Segregating phenotypes of matings made between red jungle fowl and between brown Leghorns and red jungle fowl

Type of mating			Chick down phenotypes: Wild type (e^+)			Brown (e^be^b)			Speckled head (e^be^y)			Speckled head (e^se^y)			Yellowish-white (e^ye^y)			Expected ratio	Total
No.	Sire	Dam	♂♂	♀♀	Total	♂♂	♀♀	Total	♂♂	♀♀	Total	♂♂	♀♀	Total	♂♂	♀♀	Total		
(1)	e^+e^y	e^+e^y	17	18	66*	—	—	—	—	—	—	—	—	—	3	6	19*	3:1	85
(2a)	e^+e^y	e^ye^y	—	—	6	—	—	—	—	—	—	—	—	—	—	—	6	1:1	12
(2b)	e^ye^y	e^+e^y	15	9	29*	—	—	—	—	—	—	—	—	—	18	13	33*	1:1	62
(3)	e^be^y	e^be^y	—	—	—	—	—	—	3	2	5	—	—	—	—	—	—	all	5
(4)	e^be^y	e^be^y	—	—	—	13	13	32*	13	27	50*	—	—	—	6	19	28*	1:2:1	110
(5)	e^be^y	e^be^y	—	—	—	—	—	—	9	5	15*	—	—	—	16	5	21*	1:1	36
(6)	e^be^y	e^ye^y	—	—	—	—	—	—	—	—	—	—	—	—	24	30	76*	all	76
(7)	e^be^y	e^se^s	—	—	—	—	—	—	—	—	—	1	1	2	—	—	—	all	2

* The totals are greater than the sum of the male and female totals since the sexes of some or all of the chicks were not determined.

TABLE 2

Segregating phenotypes of matings made between brown Leghorns

Chick down phenotypes

Type of mating			Wild type (e^+)			Wild type speckled head (e^+e^s)			Brown (e^be^b)			Brown head (e^be^s)			Speckled head (e^se^s)			Expected ratio	Total
No.	Sire	Dam	♂♂	♀♀	Total	♂♂	♀♀	Total	♂♂	♀♀	Total	♂♂	♀♀	Total	♂♂	♀♀	Total		
(1)	e^be^b	e^be^b	—	—	—	—	—	—	23	33	74* (a)	—	—	—	—	—	—	all	74
(2)	e^se^s	e^se^s	—	—	—	—	—	—	—	—	—	—	—	—	5	6	25*	all	25
(3)	e^+e^s	e^+e^b	18	19	46*	6	8	14	—	—	—	10	3	17*	—	—	—	2:1:1	77
(4)	e^be^s	e^be^s	—	—	—	—	—	—	25	20	51*	38	31	78*	25	20	51*	1:2:1	171
(5)	e^+e^b	e^+e^b	0	3	12*	4	2	11*	1	1	8* (b)	—	—	—	—	—	—	1:2:1	31
(6)	e^be^b	e^+e^b	—	—	—	3	3	9* (c)	4	4	14* (d)	—	—	—	—	—	—	1:1	23
(7)	e^+e^s	e^be^b	—	—	—	8	6	23* (c)	—	—	—	8	12	28* (e)	—	—	—	1:1	51
(8)	e^be^b	e^be^s	—	—	—	—	—	—	2	5	14*	2	3	12*	—	—	—	1:1	26
(9)	e^be^s	e^se^s	—	—	—	—	—	—	—	—	—	5	4	10* (f)	4	3	10*	1:1	18

* The totals are greater than the sum of the male and female totals since the sexes of some or all of the chicks were not determined.

(a) Three chicks resembled a dark e^be^b phenotype.
(b) Two chicks resembled the e^be^s phenotype.
(c) Two chicks resembled the e^+e^+ phenotype.
(d) Three chicks resembled the e^be^s phenotype.
(e) Two chicks resembled the e^be^b phenotype.
(f) Three chicks resembled the e^be^b phenotype.

TABLE 3

Segregating phenotypes of matings made between brown Leghorns (e^be^s) *and birds derived from the Australorp breed of fowl of the genotype* E e^s. *Hens of this genotype were entirely black, and* E e^s *cocks were primarily black (black breast, tail, primaries, secondaries; dark red neck and rump hackles; and maroon colored back)*

Chick down phenotypes

Type of mating			Black (*E*)†			Brown head (e^be^s)			Speckled head (e^se^s)			Expected ratio	Total
No.	Sire	Dam	♂♂	♀♀	Total	♂♂	♀♀	Total	♂♂	♀♀	Total		
(1)	*E* e^s	e^be^s	29	46	81*	15	15	33*	19	15	37*	2:1:1	151
(2)	*E* e^s	*E* e^s	28	25	72*	—	—	—	2	6	15*	3:1	87

* The totals are greater than the sum of the male and female totals since the sexes of some of the chicks were not determined.

† Most chicks were black with whitish belly and chin or throat; some chicks were entirely black, and others (4) had whitish areas and/or spots on the sides of the head. All chicks were predominantly black except for the areas mentioned.

Ee^s were employed to test allelism of *E* to genes e^b and e^s. This test was accomplished in two ways: (1) An F_2 progeny was secured from the mating *Ee*^{s} × *Ee*^{s}; (2) a cock *Ee*^{s} was mated to hens of the genotype e^be^s. Results of these matings are shown in table 3, matings (1) and (2). Results of these tests considered with results of the other tests strongly suggest a series of multiple alleles at the e^+ locus.

IV. *Linkage test with pea comb.* The dark Cornish breed of domestic fowl possessing pea comb (*P*) as a breed characteristic was locally available and was used in these tests. It has been demonstrated (see HUTT 1949 for complete data) that pea comb is linked with the gene for blue egg (*O*) and the gene for marbled down (*ma*). The gene for naked neck (*Na*) is also a member of this autosomal linkage group.

TABLE 4

Test for linkage between e^+ *(striped wild type) and* P *(pea comb). An* F_1 *female of the genotype* P/p, e^y/e^+, *derived from dark Cornish and brown Leghorn parents, was backcrossed to a red jungle fowl male* (p/p, e^y/e^y). *There is no significant deviation from a 1:1:1:1 ratio on the basis of independently assorting genes*

Segregating phenotypes

Striped wild type (e^+)						Yellowish-white or Cornish modification ($e^y e^y$)†					
Pea comb			Single comb			Pea comb			Single comb		
♂♂	♀♀	Total	♂♂	♀♀	Total	♂♂	♀♀	Total	♂♂	♀♀	Total
7	5	13*	8	4	16*	9	7	17*	9	6	16*

* The totals are greater than the sum of the male and female totals since the sexes of some of the chicks were not determined.

† See text for explanation.

Dark Cornish exhibit sexual dichromatism: Cocks are black with dark red backs and chestnut secondaries; wing coverts, neck and rump hackle feathers may have a shade of mahogany-red. Hens are penciled (chestnut feathers with black crescentic marks) on the back, breast, wings, and tail, and have black necks. Ground color of chicks in the strain used was greyish-white with irregular black or slate-grey dorsolateral back stripes and grey mid-dorsal back stripes. The latter stripes were extremely variable in outline. The dorsal neck region was striped, but this stripe was broken in several spots on the head. Other strains of dark Cornish chicks have dark brown down (KIMBALL 1952b) or wild type striped down (PEASE personal communication).

Down color of the strain of dark Cornish used was found to be basically governed by the allele e^y; however, it was subject to some modification in phenotype by other plumage genes of dark Cornish. One F_1 double heterozygous hen was used to test for linkage. She had a Cornish dam (P/P, e^y/e^y) as one parent and the sire was a brown Leghorn, single comb, striped wild type (p/p, e^+/e^+). The F_1 hen thus was presumably of the genotype P/p, e^y/e^+ (pea comb, striped wild type). This hen was backcrossed to a double recessive male red jungle fowl (p/p, e^y/e^y). Data presented in table 4 indicate that e^+ is independent of P.

THE CORNISH DOWN PATTERN

The possibility of another allele at the e^+ locus was investigated. Attempts were made to extract birds with Cornish striping as chicks and non-penciled phenotypes as adults. Ideally, if Cornish striping represented another allele of e^+, then removal of the penciling character might reveal a new mutant phenotype in the adult hen and, theoretically, phenotypically wild type cocks.

In the test for linkage (table 4) the segregating phenotypes expected (disregarding P and p) were striped wild type and Cornish striped or yellowish-white in a ratio approximating 1:1. The mutant phenotype realized, however, ranged from typical yellowish-white (e^y) to an imperfect Cornish striped phenotype. This result, however, might have been expected since (as mentioned previously) the down pattern of the

Cornish strain used was somewhat variable. Polygenic modifiers seem to provide an explanation for this variation. Chicks having an imperfect Cornish down pattern (refer to table 4) were raised to maturity. Although only one cock and one hen survived to be definitely classified as adults, the cock was phenotypically wild type and the hen showed an $e^{v}e^{v}$ phenotype. It is on this evidence that the down pattern of the dark Cornish strain studied is believed to be basically governed by the e^{v} allele.

HORMONAL CONTROL OF ADULT PHENOTYPE

In another study, the author (1953) demonstrated that adult red jungle fowl males of the genotype $e^{v}e^{v}$ and $e^{+}e^{+}$ were indistinguishable from each other; whereas female red jungle fowl of the same genotypes were phenotypically distinct. Gonadectomy of e^{+} and e^{v} males resulted in no change of phenotype in regenerating feathers. Males (e^{+} and e^{v}) each given a 15 mg pellet of a synthetic estrogen (diethylstilbestrol) under the skin of the neck, however, showed differences in phenotype of regenerating feathers. Feathers of e^{+} males approximated feathers of e^{+} females; feathers of e^{v} males approximated feathers of e^{v} females. Diethystilbestrol elicited the same response in castrates as it did in intact males.

In the present study the same method of diethystilbestrol pellet implantation was used. Adult males of the genotypes $e^{b}e^{b}$, $e^{b}e^{s}$, and $e^{s}e^{s}$, phenotypically indistinguishable (wild type) as adults, were each given a 15 mg pellet implant of diethystilbestrol. At this time feathers of the rump and breast were plucked so that new emerging feathers would grow under the influence of estrogen. Reaction responses of these cocks were as expected; new emerging feathers approximated the corresponding female adult phenotype in each instance. From these results it can be seen that estrogen (diethylstilbestrol) can be used in genotype determination of wild type males when doubt exists as to homozygosity of e^{b}, e^{s}, or e^{v}. The phenotypes of e^{+}, e^{b}, e^{s}, and e^{v} adult females are obviously only manifest under the influence of ovarian hormones.

DISCUSSION

Validity of the concept of multiple alleles of e^{+}

Tables 1, 2, and 3 present the types of matings which were made to establish allelism between *E*, e^{+}, e^{b}, e^{s}, and e^{v}. In most cases heterozygotes were phenotypically intermediate relative to their respective homozygotes. The allele e^{v} is presumed to be recessive to all other alleles, although it has not been tested with *E*. Homozygosity of each mutant phenotype ($e^{b}e^{b}$, $e^{s}e^{s}$, $e^{v}e^{v}$) is demonstrated. The mating brown Leghorn females ($e^{b}e^{b}$) $\times$ red jungle fowl male ($e^{v}e^{v}$) was made (table 1, mating 3). F_1 individuals of this mating (fig. 1, E) resembled e^{s} homozygotes to some degree. However both phenotypes could be distinguished from each other, since the hybrids ($e^{b}e^{v}$) had dark slate-brown undersides and other speckled headed chicks ($e^{s}e^{s}$) did not. In general $e^{b}e^{v}$ individuals were darker throughout and speckling of the head was more extensive. Since the e^{v} homozygote tends to produce an extreme dilution of buff and brown pigment and the e^{b} homozygote, conversely, tends to darken down color, the allelic compound ($e^{b}e^{v}$) in down color below the head approaches and very nearly duplicates the wild type dorsal back striping. It is not as light as the wild type nor as dark as the mutant $e^{b}e^{b}$. Incomplete or no dominance, however, prevails in the head

region of the compound. As mentioned previously, an irregularly shaped dorsal head stripe is found with dark brown spots and streaks along the lateral sides of the head. From this it can be seen that the action of e^b is to extend brown pigment over the head, for it is in this region that intermediacy is found. As adults $e^b e^y$ hens resemble the $e^b e^s$ phenotype.

Brown Leghorn heterozygotes ($e^b e^s$) were intermediate in phenotype between e^b and e^s homozygotes, whereas $e^+ e^b$ heterozygotes (= speckled head wild type) usually differed from wild type only by the presence of small spots between the dorsal head stripe and the eye stripe. In one mating (table 2, mating 6) an occasional wild type individual was produced, which indicated that the threshold of phenotypic change from wild type to speckled head wild type is probably under polygenic control so that e^+ acts as a complete dominant. A similar situation exists in the $e^b e^s$ heterozygote. In most matings this phenotype (called brown head, fig. 1, F) was clearly recognizable, however, occasional individuals resembled e^b homozygotes (table 2, mating 7). Conversely, in matings of $e^b e^b$ *inter se* individuals may approach and almost duplicate the brown head phenotype. The allele e^s is apparently completely recessive to e^+.

As adults, $e^b e^s$ individuals were intermediate in phenotype: greyish-brown stippled breasts and backs. Adult individuals of the genotype $e^+ e^b$ and $e^+ e^s$ were wild type (breasts were non-stippled salmon-colored).

The allele e^b in an $e^+ e^b$ combination may act as a complete recessive, for an occasional $e^+ e^b$ chick is phenotypically wild type as in mating (6). It may therefore be said that the e^b allele occasionally lacks "penetrance". Polygenic modifiers apparently are present and in matings involving alleles e^+ and e^b segregation of $e^b e^b$ zygotes may not always be apparent. In mating (5) presumed homozygotes ($e^b e^b$) resembled the $e^b e^s$ phenotype. The converse may also be true in matings where brown heads ($e^b e^s$) are expected. In this case $e^b e^s$ zygotes may resemble $e^b e^b$ zygotes as in mating (7).

A mating between a homozygous speckled head ($e^s e^s$) brown Leghorn female and a red jungle fowl mutant male ($e^y e^y$) produced F_1 chicks which had the speckled head phenotype (table 1, mating 7). This mating indicated that e^y was recessive to e^s.

The relationship between E, e^b, and e^s was adequately demonstrated in matings (1) and (2) of table 3. Mating (1) alone, however, cannot establish allelism of E, e^b, and e^s since the 2:1:1 ratio can also be produced with independently assorting non-allelic genes. Since the same cock used in mating (1) was mated with his sibling of identical ancestry and in F_2 produced a 3:1 segregation of black to speckled head, it indicates that at least e^s is allelic to E. Considering the known relationship of E to e^+ and the relationship of e^b, e^s, and e^y to e^+, it follows indirectly that E, e^+, e^b, e^s, and e^y can be considered to represent a series of multiple alleles. The relationship between e^y and E has not been determined. Arranged in order of decreasing dominance the alleles may be listed as follows: E-e^+-e^b-e^s-e^y.

The genotypes postulated for the sires and dams in tables 1, 2, and 3 were determined, when possible, from the parentage and phenotypes of the birds crossed and from segregation observed in their progeny. In most cases parentage and progeny segregation from diallel crosses were the main criteria used for genotypic determination. However, where known homozygous strains were used, homozygosity for the

particular allele was assumed. Individual birds used in crosses involving ratios of 2:1:1 were always used in crosses involving different combinations of alleles, i.e., table 2, mating 3, sire e^+e^s was used in mating 7; dam e^+e^b was used in mating 6. Likewise, in table 3, sire Ee^s was used in both matings, and dam e^be^s was used in mating 4 of table 2. In most of the other matings the same method of "overlapping" sires and dams in different matings was employed. All data yielded results consistent with expectation.

Many other domestic breeds possibly carry other mutants of e^+. The only evidence of the occurrence of the mutant e^b in the wild state is a female red jungle fowl collected in February, 1908 by L. L. KRIBS near Illigin on the Island of Mindanao, Philippine Islands (University of California, Museum of Vertebrate Zoology Number 30947). It has the adult e^b phenotype (dark brown stippled breast) and presumably is of the genotype e^be^b.

Primary and secondary plumage pattern genes

KIMBALL (1953a) defined a primary pattern gene as one determining ". . . pterylar and multipterylar distribution of specific black feather pigments, *viz.*, e^+, e, and E." He defines a secondary pattern gene, such as stippling (Sg), ". . . as a factor determining distribution of black pigment within the individual feather." This classification of gene action on pigment distribution is useful in specific instances, but it necessarily becomes useless when attempts are made to apply it to a series of multiple alleles. Gene action in relation to color deposition can be classified in several ways (dilutions, extensions, inhibitors or suppressors, etc.), and the fact that the potentialities of alleles of one locus can be extremely variable (affecting the same character in different ways) has been shown by studies on other forms (CASTLE 1930, 1940; GRUNEBERG 1943). Well known examples are the alleles at the C^+ locus in domestic rabbits. Here the mutant chinchilla (c^{ch}) can be classed as a secondary pattern gene, whereas Himalayan (c^H) and albino (c^a) can be considered primary pattern genes. Considering the mutants of e^+, the allele e^y can be classified as a dilution gene. Alleles e^b and e^s, although producing nearly identical adult phenotypes, are clearly distinct in their action on down plumules. These can be classified as secondary pattern genes. From the evidence submitted it follows that the author disagrees with the classification of primary and secondary pattern genes, postulated by KIMBALL, as applicable to a series of multiple alleles.

SUMMARY

1. Evidence supporting the concept of multiple alleles in the fowl is presented.

2. Descriptions and relationships of three mutants, e^b (brown), e^s (speckled head) and e^y (yellowish-white) to E (extended black) and e^+ (wild type striped) are discussed.

3. Evidence presented demonstrates no linkage with P (pea comb).

4. All mutants with the exception of E affect both sexes as chicks, but as adults, cocks are not affected and hens demonstrate the mutant phenotype.

5. Down pattern in the strain of dark Cornish used is believed to be basically governed by allele e^y.

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TRANSLOCATIONS IN MAIZE INVOLVING CHROMOSOME 6

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CHROMOSOME 6 of maize may be characterized by the presence of the nucleolus organizer region (and the small portion beyond, known as the satellite). Its association with the *Y-Pl* (yellow endosperm-1, purple plant color) linkage group was established by McCLINTOCK (1931) by a study of trisomic plants and by means of a deficiency in the long arm which showed the orientation to be nucleolus-*Y-Pl*. BRINK and COOPER (1932) presented linkage data for *T1-6a* (semisterile-5) and established the order nucleolus-*Y-Pl-T* for this translocation.

The presence of *Y* and the nucleolus organizer region has made translocations involving chromosome 6 particularly valuable as tools in a number of important studies. Evidence was provided by *T1-6b* (BURNHAM 1932) that centromere disjunction is reductional at anaphase I of meiosis. BURNHAM (1949, 1950) used the nucleolus organizer to show that the frequency of disjunction types in translocations is closely related to lengths of interstitial segments. The close linkage of *Y* with *su* (sugary endosperm-1) on chromosome 4 in *T4-6a* (ANDERSON 1952) and the early appearance of yellow color was used by TEAS *et al.* (1952) to separate developing sugary and starchy kernels on segregating ears for a comparative study of tryptophan, niacin, indole acetic acid, and carbohydrates during their ontogeny.

In studies illustrated by the foregoing examples as well as studies to localize sections of chromosomes associated with such characters as smut resistance (BURNHAM and CARTLEDGE 1939), leaf firing (SABOE and HAYES 1941), and resistance to attack by the European corn borer (IBRAHIM 1954), it is important to know the positions of the breaks and their linkage relations with genes whose relative positions on the linkage maps are known.

The present paper summarizes the available information for those translocations involving chromosome 6 to which permanent numbers have been assigned.

CYTOLOGICAL POSITIONS AND PREVIOUS INFORMATION

Forty-six translocations involving chromosome 6 are listed serially in table 1. Cytological re-examinations of the break positions were made from material grown in 1954 and the positions are given in this table as the fraction of the distance from the centromere to the end of the arm involved. Thus 6L.82 indicates a break far out on the long arm of chromosome 6 while 6S.09 indicates a break near the centromere on the short arm. These positions supplant those which may have been given in earlier publications. References are given in this table to the principal papers in which the translocations previously have been studied or used. Attention should be

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[2] Journal paper No. 828 of the Purdue University Agricultural Experiment Station.

TABLE 1

List of translocations involving chromosome 6, cytological positions, and references to previous studies

Translocation	Cytological positions		Reference*	Translocation	Cytological positions		Reference*
T1-6a	1L.21	6L.59	1, 9, 11, 12	*T5-6a*	5L.32	6L.47	1, 11
T1-6b†	1L.25	6S[s]	1, 10, 11, 13	*T5-6b*	5L.71	6L.29	11
T1-6c	1S.17	6L.39	4, 11	*T5-6c*	5L.81	6S.11	11
T1-6d	1L.10	6S.71[o]	11, 15	*T5-6e*	5L.20	6L.63	
T1-6e	1S.37	6L.40		*T5-6f*	5S.29	6S.70[o]	
T1-6f	1L.35	6L.45					
T1-6g	1L.16	6L.84		*T6-7a*	6L.74	7L.61	
T1-6h	1L.05	6L.16		*T6-8a*	6L.41	8L.73	1, 3, 11, 12
				T6-8b	6L.73	8S.72	
T2-6a	2S.51	6S.09	1, 11, 12	*T6-8c*	6L.29	8L.50	
T2-6b	2S.69	6L.49	1, 14	*T6-8d*	6L.51	8L.78	11
T2-6c	2L.32	6L.20	1, 11, 14	*T6-9a*	6S.79[o]	9L.40	1, 2, 13, 14, 16
T2-6d	2L.41	6L.45	1, 11	*T6-9b*	6L.13	9S.42	1, 2, 11
T2-6e	2L.28	6L.22	1	*T6-9c*	6L.17	9L.20	11
T2-6f	2L.78	6L.87		*T6-9d*	6S.81[o]	9L.86	14
				T6-9e	6L.17	9L.22	14
T3-6a	3L.08	6L.26	1, 6, 11	*T6-10a*	6L.68	10L.19	1, 7, 11, 12, 14
T3-6b	3S.70	6S.77[s]	1, 6, 11	*T6-10b*	6L.18	10L.21	7, 11
T3-6c	3S.56	6L.54	11, 14, 15	*T6-10c*	6L.51	10S.40	
T3-6d	3L.28	6L.83		*T6-10d*	6L.15	10L.06	
				T6-10e	6L.15	10S.15	
T4-6a	4L.33	6L.44	1, 5, 8, 11, 16	*T6-10f*	6S.92[s]	10S.30	
T4-6b	4S.79	6L.14	1, 8, 11	*T6-10g*	6L.86	10L.19	
T4-6c	4S.29	6S.83[s]	1, 8	*T6-10h*	6L.48	10L.86	
T4-6d	4L.46	6L.56	15				
T4-6e	4S.70	6L.60	8, 14				

* 1, 2, 3, 4, 5, ANDERSON 1935, 38, 39, 41, 52; 6, ANDERSON and BRINK 1940; 7, ANDERSON and KRAMER 1954; 8, ANDERSON, KRAMER, and LONGLEY 1955; 9, BRINK and COOPER 1932; 10, 11, BURNHAM 1932, 50; 12, BURNHAM and CARTLEDGE 1939; 13, EMERSON, BEADLE, and FRASER 1935; 14, IBRAHIM 1954; 15, ROBERTS 1942; 16, SABOE and HAYES 1941.

† Position by BURNHAM 1932.

[s] Satellite. [o] Nucleolus organizer.

called specifically to those translocations reported under different designations than the permanent numbers assigned here. These are *T1-6a*, reported first as semisterile-5 (BRINK and COOPER 1932) *T1-6b*, reported as a low sterile by BURNHAM (1932), *6-8d*, previously *6-8D-1* and *6-9c*, previously *6-9A66* (BURNHAM 1950) and three reported by ROBERTS (1942), namely, *T1-6d* (*Conn.R28*), *3-6c* (*Conn.R34*), *4-6d* (*Conn.R43*). Certain translocations are reported here for the first time. They were obtained by X-ray treatment as described by ANDERSON *et al.* (1955).

LINKAGE DATA

Linkage tests were made with the genes *Y*, *Pl*, *sm* (salmon silks), and *py* (pigmy plant). The position of these four genes on the linkage map given by RHOADES (1950) is

$$\frac{Y \quad Pl \quad sm \quad py}{13 \quad 44 \quad 54 \quad 64}.$$

TABLE 2

Three-point linkage tests of translocations involving chromosome 6

Translocation	Genotypic group	Parental		Recombination in region						Total	Percent recombination	
				1		2		1, 2			Region 1	Region 2
Genotypic groups 1, *Y + Pl/ + T +*; and 2, *Y ++ / + T Pl*												
T1-6c	1	90	86	0	2	13	9	0	0	200	1.0	11.0
T3-6a	2	66	47	1	7	3	0	2	0	126	7.9	4.0
T4-6c	1	50	67	0	0	14	21	0	0	152	0.0	23.0
T6-8a	1	45	33	7	4	0	0	0	0	99	11.1	0.0
	1	47	63	7	6	0	15	1	0	139	10.1	11.5
T6-9b	1	144	180	0	1	6	14	0	0	345	0.3	5.8
	1	49	107	1	3	6	2	1	1	170	3.5	5.9
Genotypic groups 3, *+ Pl sm/T ++*; and 4, *++ sm/T Pl +*												
T1-6c	3	211	205	4	11	5	3	0	0	439	3.4	1.8
T2-6a	4	121	43	7	11	3	3	0	0	188	9.6	3.2
T2-6c	3	117	134	9	4	10	6	0	1	281	5.0	6.0
T2-6d	3	48	36	2	8	2	2	0	3	101	12.9	6.9
T2-6e	3	84	86	3	8	5	2	0	1	189	6.3	4.2
T3-6a	3	93	108	2	4	6	6	0	0	219	2.7	5.5
T4-6b	3	62	—	—	2	—	1	0	—	65	3.1	1.5
	3	124	—	11	—	15	—	0	—	150	7.3	10.0
T4-6c	3	156	65	124	18	19	3	4	1	390	37.7	6.9
T6-8a	3	102	79	1	2	2	1	0	2	189	2.6	2.6
T6-9b	3	190	263	14	18	8	9	1	4	507	7.3	4.3
T6-10b	4	60	61	8	4	4	1	0	0	138	8.7	3.6
Genotypic group 5, *+ sm + /Pl + T*												
T2-6b	5	350	320	27	28	14	11	3	0	753	7.7	3.7
T6-10a	5	138	146	13	13	37	43	2	1	393	7.4	21.1
Genotypic group 6, *++ py/T Pl +*												
T3-6a	6	24	47	1	1	6	2	1	0	82	3.7	11.0
Genotypic group 7, *++ py/sm T +*												
T6-10a	7	—	82	14	—	3	—	—	1	100	15.0	4.0

Results of three-point backcross tests are presented in table 2 for 13 translocations. Tests were made with F_1 plants which can be grouped into 7 genotypes. For example, genotypic groups 1 and 2 are alike in that they include translocations in which the order was *Y-T-Pl*, but they differ in linkage phase. The genotypic group is indicated in the second column of the table. The order of presentation of the data follows that of EMERSON *et al.* (1935) and the recombination values for both regions are in the last two columns.

In many instances, particularly in the preliminary stages of determining the

TABLE 3

Summary of two-point linkage tests

Translocation	Region					
	T-Y			*T-Pl*		
	Number	Recombinations	Percent	Number	Recombinations	Percent
T1-6c	405	4	1.0	404	22	5.4
T1-6g	—	—	—	154	36	23.4
T1-6h	258	9	3.5	—	—	—
T2-6b	—	—	—	401	47	11.7
T2-6d	—	—	—	208	3	1.4
T2-6e	170	8	4.7	156	6	3.8
T3-6a	219	13	5.9	581	16	2.8
T3-6b	231	36	15.6	219	57	26.0
T4-6b	1179	66	5.6	—	—	—
T4-6c	345	42	12.2	97	27	27.8
T5-6a	—	—	—	113	0	0.0
T5-6b	167	32	19.2	160	55	34.4
T5-6c	—	—	—	207	20	9.7
T5-6f	38	4	10.5	—	—	—
T6-8a	371	40	10.8	—	—	—
T6-9c	542	2	0.4	286	13	4.5
T6-9e	269	0	0.0	—	—	—
T6-10b	—	—	—	236	18	7.6

chromosomes involved in a translocation, two-point tests were made either with *Y* or *Pl*. These additional data are presented in table 3.

SUMMARY OF INDIVIDUAL TRANSLOCATIONS

The cytological positions given in table 1, the three-point data in table 2, the additional two-point data presented in table 3, and previously published data, can be used to arrange the translocations in the approximate order of the position of their breaks from left to right on chromosome 6. Such an arrangement is suggested in table 4. In a few instances, linkage data from homozygous interchange stocks were obtained to aid in establishing the order more definitely. The following brief summary for each translocation indicates the extent and limitations of the available data.

T1-6a. The break is in the distal half of the long arm of 6. Three-point data of BRINK and COOPER (1932) place it 8.2 units to the right of *Pl*.

T1-6b. BURNHAM (1932) has determined the break in 6 to be in the satellite region. His recombination values of *T*-13.8-*Y* and *T*-44.9 *Pl* (from EMERSON *et al.* 1935) agree in placing it well to the left of *Y*.

T1-6c. In spite of some inconsistency in the *T-Pl* recombinations from two 3-point tests in table 2, the break definitely appears to be to the left of *Pl* and near *Y*. ANDERSON (1941) placed the break on 1 to the right of *P*. The positions at 1S.2 and 6L.4 would indicate the break to be proximal both to *P* and to *Pl*. This is confirmed in the homozygous translocation where *P* showed 46.4 percent recombination with *Pl* which for 293 plants is not significantly different from independence.

TABLE 4

The approximate order from left to right of chromosome 6 translocations

Translocation	Gene order and recombination							Position on chromosome 6
T3-6b	*T*	15.6 (231)	*Y*					S.77*
*T1-6b**	*T*	13.8 (130)	*Y*					S*
T4-6c	*T*	8.4 (479)	*Y*	23.0 (152)	*Pl*	6.9 (390)	*sm*	S.83°
T6-9a†	*T*	12.9 (402)	*Y*	22.4 (402)	*Pl*			S.79°
T5-6f	*T*	10.5 (38)	*Y*					S.70°
T5-6c	*T*			9.7 (207)	*Pl*			S.11
T2-6a	*T*			9.6 (188)	*Pl*			S.09
T4-6b	*T*	5.6 (1098)	*Y*	9.3 (290)	*Pl*	10.3 (290)	*sm*	L.14
T1-6h	*T*	3.5 (258)	*Y*					L.16
T6-10b	*T*			8.2 (417)	*Pl*	3.6 (138)	*sm*	L.18
T6-9b	*Y*	1.4 (515)	*T*	5.5 (2218)	*Pl*	4.3 (507)	*sm*	L.13
T6-9c	*Y*	0.4 (542)	*T*	4.5 (286)	*Pl*	4.2 (189)	*sm*	L.17
T6-9e	*Y*	0.0 (269)	*T*					L.17
T2-6c			*T*	5.0 (281)	*Pl*	6.0 (281)	*sm*	L.20
T2-6e	*Y*	4.7 (170)	*T*	5.2 (345)	*Pl*	4.2 (189)	*sm*	L.22
T1-6c	*Y*	1.0 (605)	*T*	5.7 (1043)	*Pl*	1.8 (439)	*sm*	L.39
T4-6a‡	*Y*	1.3 (389)	*T*	5.3 (1025)	*Pl*	5.5 (325)	*sm*	L.44
T3-6a	*Y*	6.7 (345)	*T*	3.1 (1007)	*Pl*	5.5 (219)	*sm*	L.26
T6-8a	*Y*	11.3 (789)	*T*	4.9 (427)	*Pl*	2.6 (189)	*sm*	L.41
T2-6d			*T*	5.2 (309)	*Pl*	6.9 (101)	*sm*	L.45
T5-6a			*T*	0.0 (113)	*Pl*			L.47
T1-6a§	*Y*	39.8 (98)	*Pl*	8.2 (98)	*T*			L.59
T2-6b			*Pl*	7.7 (753)	*sm*	3.7 (753)	*T*	L.49
T1-6g			*Pl*	23.4 (154)			*T*	L.84
T6-10a	*Pl*	9.5 (493)	*sm*	17.0 (100)	*py*	3.0 (134)	*T*	L.68

* Data of C. R. BURNHAM from EMERSON *et al.* (1935).
† Data of ANDERSON (1934).
‡ Data of ANDERSON (1952).
§ Data of BRINK and COOPER (1932).
* Satellite. ° Organizer.

T1-6g. The position at 6L.84 and 23.4 percent recombination for *T-Pl* suggest the break to be well beyond *Pl*.

T1-6h. The data in table 3 indicate only that the translocation is in the general region of *Y*.

T2-6a. Cytological placement is 6S.09. The order *T*-9.6-*Pl-sm* is in agreement.

T2-6b. The break at 6L.49 is definitely to the right of *sm*.

T2-6c. The interchange is at 6L.20 and 5 units to the left of *Pl*.

T2-6d. The three-point test in table 3 gave the order *T-Pl-sm*. Cytological positions are in the long arms both of 2 and 6. Extensive tests with the homozygous translocations showed independence of *Pl* and v_4 on the long arm of two (49.3 percent recombination for 1265 plants), but linkage of *Pl* with *B* on the short arm of 2. (36.4 percent, 1656 plants). These results confirm the placements on the long arms of both chromosomes with breaks proximal both to *Pl* and v_4.

T2-6e. The conclusions are similar to *T2-6d*. Data for the heterozygous translocation indicate the order *T-Pl-sm*. Cytological positions are on the long arms of both

chromosomes. In the homozygous translocation there was 50.8 percent (222/437) recombination for *Pl-v*$_4$ and 12.2 percent (20/164) for *Pl-B*.

T3-6a. Data from 3 different genotypes in table 2 agree in placing the interchange to the left of *Pl*. In genotype 2, the *Y* + +/+ *T Pl* plant was used as the pollen parent and it is possible that some pale yellow seeds were classified as white, thus inflating one crossover class in region 1. The interchange, therefore, while it appears to be to the left of *Y* can not be established with certainty from these data.

T3-6b. The interchange is in the satellite region and the two-point data in table 3 agree in placing it well to the left of *Y*.

T4-6a. ANDERSON (1952) has completely characterized this translocation. The break is at or very near *Y*, possibly to the left.

T4-6b. Partial 3-point data are available on two cultures. In one, difficulty was experienced in classifying for *sm* and only data from 65 *sm* plants are considered reliable. In a second culture, a marked deficiency of semisterile plants was noted and the data from 150 normal plants are presented. The larger culture more definitely places the interchange to the left of *Pl* but the recombination values are probably inflated because of the excess of non-semisterile plants. The 2-point data with *Y* in table 3 also were found to be somewhat variable with values ranging from less than 2 to more than 7 percent. The interchange is probably to the left of *Pl* but its position relative to *Y* is uncertain.

T4-6c. Both cytological and genetic data place the break well to left of *Y*.

T5-6a. With the interchange at 6L.47, no crossover in the *T-Pl* region was observed among 113 plants.

T5-6c. Cytological placement is on the short arm but near the centromere.

T5-6f. Although only 38 plants were tested, 10.5 per cent recombination for the *T-Y* region is consistent with the cytological placement on the short arm.

T6-8a. The interchange appears to be to the right of *Y* but its position with respect to *Pl* remains uncertain. In the homozygous translocation, *Y* shows 16.1 percent (20/124) recombination with *j* on the long arm of 8. Since ANDERSON (1939) gives the order *T*-7.8-*ms*$_8$-*j* for chromosome 8 this translocation is distal to *Y* and proximal to *j*.

T6-9a. The break on chromosome 6 is in the nucleolus organizer region. ANDERSON (1934) showed the order to be *T-Y-Pl*. In 3-point backcross tests with the F_1 used as a female his values were *T*-4.9-*Y*-16.2 *Pl* for 142 plants, while for the F_1 used as a male the values were *T*-17.3-*Y*-25.8 *Pl* for 260 plants. The higher values appear more in line with the break in the organizer region.

T6-9b. The position appears definitely to be between *Y* and *Pl*. In homozygous tests *Pl* and *sm* remained linked. *Pl* and *wx* are independent in the homozygote showing that the interchange at 6L.13, 9S.42 is proximal both to *Pl* on 6 and *wx* on 9.

T6-9c. The data in table 3 indicate only that the break at 6L.17 is near *Y*.

T6-9e. With the break at 6L.22, no recombinants with *Y* were found in 269 plants.

T6-10a. At 6L.68, the break is definitely to the right of *sm*. In the homozygous translocation *Pl* and *sm* remain linked (7 recombinants in 78 plants). Partial data in table 2 in which data from the non-pigmy plants was considered reliable indicates that the break is probably beyond *py*.

T6-10b. The break at 6L.18 is proximal to *Pl*.

SUPPRESSION OF RECOMBINATION IN CHROMOSOME 6

In table 4 it will be observed that all translocations in which the interchange is known to be between *Y* and *Pl* show less than 10 percent recombination of *Y* with *Pl*. One possible exception is *T6-8a* but here the point of interchange with respect to *Pl* is uncertain. This reduction in recombination makes translocations involving chromosome 6 particularly valuable since *Y* is a non-defective readily classified endosperm character.

SUMMARY

A recent revision of the interchange positions of 46 translocations involving chromosome 6 is presented in table 1.

Three-point linkage data are presented for 13 translocations and additional two-point data are included for certain of these and for 8 additional translocations. These 21 together with 4 previously described translocations have been arranged in their approximate order from left to right on chromosome 6.

All translocations in the proximal half of the long arm show low recombination values with *Y*, and those between *Y* and *Pl* reduce the *Y-Pl* recombination from the standard map distance of 31 units to less than 10 percent.

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A CYTOGENETIC STUDY OF NATURAL AND CONTROLLED HYBRIDS BETWEEN AGROPYRON TRACHYCAULUM AND HORDEUM JUBATUM

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VIGOROUS but sterile plants identifiable as *Elymus macounii* Vasey or in some cases *Agropyron saundersii* (Vasey) Hitchc. were frequently observed in northern Utah. The morphology of these plants and the species associated with them together with their sterility indicated that they might be F_1 hybrids between *Agropyron trachycaulum* (Link) Malte and *Hordeum jubatum* L. Such a possibility was suggested by STEBBINS *et al.* (1946b). The present paper reports the results of our investigations on the cytology, comparative morphology, origin and taxonomic status of these plants.

MATERIALS AND METHODS

Anthers of the suspected hybrids and putative parents were fixed in 3:1 acetic-alcohol. Those of the controlled hybrids were fixed in NEWCOMER's (1953) solution which proved superior to the acetic-alcohol. Observations and photographs were taken from acetocarmine smears in temporary mounts.

Three crossing techniques were utilized: hand emasculation, hot water emasculation and mass pollination without emasculation or bagging; the first two methods were ineffectual. Approximately 2000 florets were emasculated and bagged with pollinator culms in vials of water during three successive field seasons without success. Hand pollination following emasculation was likewise ineffectual. Hot water emasculation at 47°C as described by KELLER (1944) was not successful. Higher temperatures were not attempted.

The successful crosses were made by surrounding a single culm of the plant to be used as the female parent with hundreds of pollinator culms placed in jars of water and changed three times at two-day intervals. All of the seed produced was planted and the plants grown to anthesis when the hybrids could be easily detected from the non-hybrids. Three hybrids were obtained using *A. trachycaulum* as the female parent and two using *H. jubatum* as the female parent. The reciprocal crosses were essentially indistinguishable.

Herbarium specimens were prepared of representative field and experimental plants and are deposited in the Intermountain Herbarium at Utah State Agricultural College.

CYTOLOGY OF THE PARENTS AND HYBRIDS

A. trachycaulum and *H. jubatum* are both tetraploids and in all probability, allotetraploids. Fourteen bivalents were consistently observed at diakinesis and metaphase I. The meiotic divisions were without abnormalities. Both species are highly

self-fertile. Twenty-eight chromosomes have been repeatedly reported for these species (MYERS 1947; COVAS 1948; others). NIELSEN (1939) reported 28 chromosomes in a root tip count of *Elymus macounii*.

The meiotic chromosome behavior of the natural and controlled hybrids between *A. trachycaulum* and *H. jubatum* is summarized in table 1. That the sterility of the hybrids is due primarily to the failure of the two complements to undergo normal

TABLE 1

Comparative meiotic chromosome behavior of natural and controlled hybrids between Agropyron trachycaulum *and* Hordeum jubatum

Chromosome association diakinesis and meta. I				No. cells	Laggards per cell ana. I	No. cells	Laggards per dyad ana. II	No. cells	No. micronuclei per pollen gr.	No. cells	No. micronuclei per tetrad	No. tetrads
I	II	III	IV									
A. Natural hybrids												
14	7			16	0	6	0	2	0	1	12	2
12	8			4	1	7	2	2	1	3	14	3
16	6			4	2	5	3	12	2	14	15	4
10	9			1	3	6	4	14	3	27	16	3
10	8			1	4	7	5	12	4	23	17	4
13	7			1	5	4	6	10	5	18	18	1
20	4			1	6	4	7	12	6	25	19	4
14	5		1	1	7	2	8	11	7	14	20	2
11	7	1		1	8	2	9	2	8	4	21	1
					10	1	10	2	9	1	22	2
					11	1	11	2	10	3	23	1
											25	2
											27	2
											30	1
B. Synthesized hybrids (combined data reciprocal crosses)												
18	5			19	3	1	4	1	0	4	3	1
14	7			13	6	1	7	1	1	37	5	1
16	6			11	10	1			2	27	6	3
20	4			7	12	1			3	21	7	9
12	8			7	13	1			4	8	9	4
16	4		1	6					5	5	10	1
14	5		1	4					6	2	11	3
16	5			2							12	3
18	3		1	2							13	1
15	5	1		2								
10	9			2								
20	5			2								
17	4	1		1								
8	7	2		1								
10	6	2		1								
8	8		1	1								
14	6		1	1								
26	1			1								
24	2			1								
22			1	1								

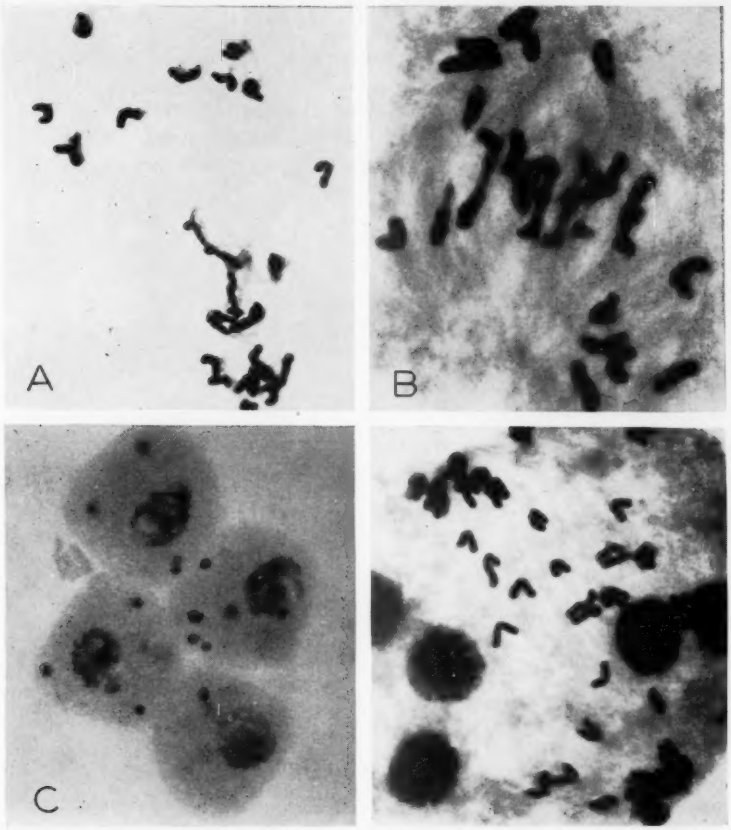

FIGURE 1.—Meiosis in the natural hybrid. A: metaphase I; 7 II, 14 I; 618×. B: metaphase I; 8 II, 12 I; 1180×. C: tetrad with micronuclei; 900×. D: anaphase I with laggards; 1030×.

pairing is apparent (fig. 1, 2). All pollen mother cells examined showed numerous unpaired univalents at diakinesis and metaphase I. These ranged from 10 to 20 with a mean of 13.8 in the natural hybrids. Univalents in the controlled hybrids ranged from 8 to 22 with a mean of 17.1. Multivalents were more frequently found in the controlled hybrids. One trivalent and one quadrivalent were observed in the natural hybrids compared with six trivalents and six quadrivalents in the controlled hybrids. In the hybrids 89 percent of the cells observed contained at least 10 chromosomes associated in pairs or occasionally with one or two multivalents. They averaged 6.3 bivalents per cell. Chromatid bridges were very rarely observed. Eighty-nine percent of the bivalents in the controlled hybrids were rods compared with 83 percent in the natural hybrids.

Practically every pollen grain of both natural and controlled hybrids contained

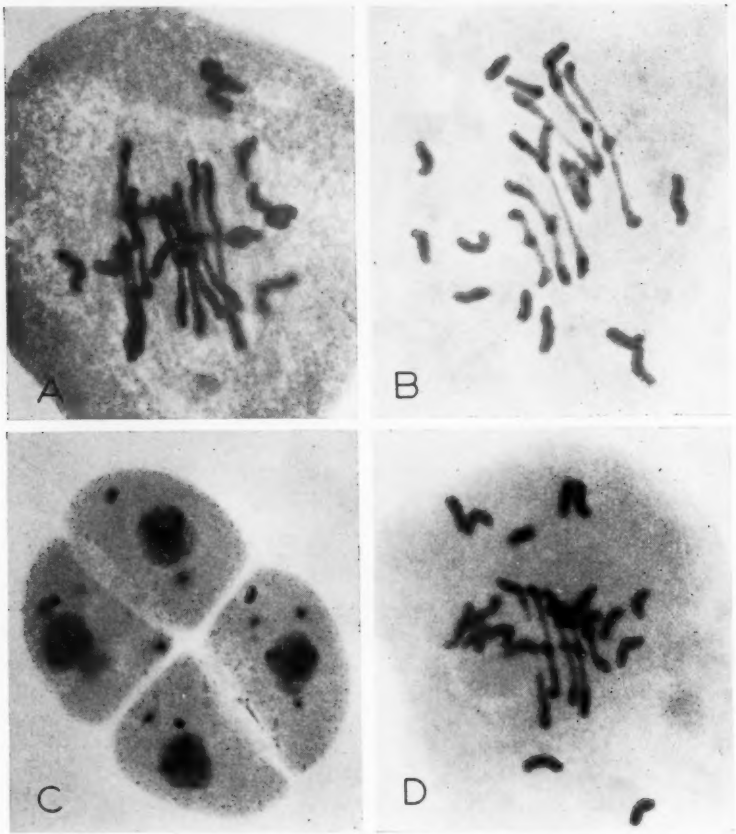

FIGURE 2.—Meiosis in the controlled hybrid. A: metaphase I; 8 II, 12 I; 827×. B: metaphase I; 1 IV, 5 II, 14 I; 1054×. C: tetrad with micronuclei; 1114×. D: metaphase I; 5 II, 18 I; 1090×.

micronuclei (fig. 1 C, 2 C). The large micronuclei probably represent anaphase I laggards which failed to be included in the new nuclei and the small micronuclei having their origin in anaphase II laggards. Practically 100 percent of the pollen appeared abortive in both natural and controlled hybrids.

The minor differences in chromosome associations between natural and controlled hybrids can probably be explained on the basis of different size samples studied. The meiotic divisions in general are definitely comparable.

MORPHOLOGY OF THE PARENTS AND HYBRIDS

The *Hordeum jubatum* found in northern Utah shows very little variation and is morphologically similar to plants found throughout the Intermountain region.

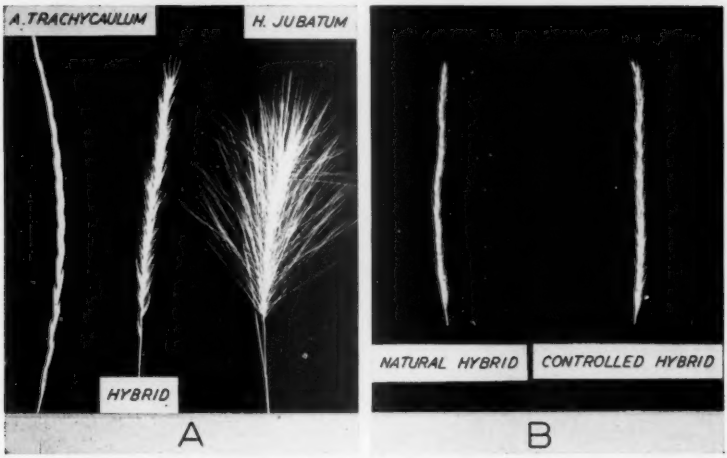

FIGURE 3.—A: spikes of *Agropyron trachycaulum*, F_1 hybrid, and *Hordeum jubatum*. B: spikes of natural and controlled hybrid.

Plants which were associated with the hybrids (herb. no. 8139) and which served as the parent plants were collected northwest of Logan in Cache County.

Agropyron trachycaulum is a highly variable species as the long synonomy list suggests (*Hitchcock* 1950). Several strains are to be found in northern Utah but plants associated with the hybrids and *H. jubatum* were rather consistent in their morphological characteristics (herb. no. 8142). These plants were generally shorter than cultivated strains, being 50–65 cm tall with spikes from 12–15 cm long and closely imbricated spikelets. Plants of this strain were successfully used as parents in reciprocal crosses.

The hybrids are more or less intermediate between the two parents (fig. 3 A). The rachis in *H. jubatum* disarticulates readily while the rachis in *A. trachycaulum* is continuous; the rachis in the hybrid disarticulates rather tardily. The lemmas in *H. jubatum* have awns 4-5 cm long while the lemmas in *A. trachycaulum* are awnless. Awns of the lemmas in the hybrid are 6–9 mm long. The hybrid differs from either of its parents in that the plants are taller (60–80 cm) and often stand 10–15 cm above the taller parent, *A. trachycaulum*.

The characteristic morphology found in natural hybrids was duplicated in the controlled hybrids and they are essentially indistinguishable (fig. 3 B). Natural hybrids with one spikelet per node are more common than those with two spikelets per node. Plants with one spikelet at a node usually have two glumes but three-glumed spikelets are common. The third glume is placed laterally on the spikelet on the side away from the rachis and is scabrous and narrow. The lemmas are usually glabrous or slightly scabrous towards the apex. The rachilla is often somewhat distorted at the base as in Elymus but otherwise the plants could be placed in either Elymus or Agropyron. Our hybrids, natural and controlled, would be referred to *E. macounii* in the Manual of Grasses (HITCHCOCK 1950).

DISCUSSION

STEBBINS, *et al.* (1946) stated that the type of *A. saundersii* "is probably an F_1 hybrid between *A. pauciflorum* (*A. trachycaulum*) and *Sitanion hystrix*." They also concluded that naturally occurring hybrids between *A. pauciflorum* and *Sitanion jubatum* correspond to *A. saundersii*. The same authors (1946b) stated that the presumed natural hybrid between *A. pauciflorum* × *H. nodosum* fits the description of *E. macounii*. They conclude finally that "the most likely hypothesis is that most specimens classified as *E. macounii*. . .are a collection of F_1 sterile hybrids between species of Agropyron and either *H. jubatum* or *H. nodosum*". KELLER (1948) stated that *A. saundersii* is the natural hybrid between *A. trachycaulum* and *Sitanion jubatum* and *Elymus macounii* is the natural hybrid between *A. trachycaulum* and *H. jubatum*.

However, so far as the present authors are aware the actual synthesis of plants which can be identified as *A. saundersii* and *E. macounii* has not previously been made nor has the meiotic chromosome behavior of either of these entities been reported. Our investigations on the meiotic chromosome behavior, comparative morphology and field observations of the parents and both natural and controlled hybrids demonstrate that at least some entities identifiable as *E. macounii* are simply F_1 sterile hybrids between *A. trachycaulum* and *H. jubatum*. These plants are therefore treated as X *Agrohordeum macounii* (Vasey) Lepage (LEPAGE 1952). Sufficient evidence to clarify the status of *A. saundersii* is not yet available.

The relative ease with which species in the genera Agropyron, Hordeum, Elymus Sitanion, Secale and Hystrix can form "intergeneric" hybrids makes possible the production of F_1 plants which may be morphologically similar yet have different origins (STEBBINS *et al.* 1946, 1946b; BRINK, COOPER and AUSHERMAN 1944).

The nature of pairing in the hybrids is difficult to completely establish. The possibility of autosyndesis cannot be completely dismissed as has been recently emphasized by STEBBINS and PUN (1953). However, the complete absence of quadrivalents in either *H. jubatum* or *A. trachycaulum* in our observations and those of other investigators argues against this interpretation here. Allosyndesis between chromosomes of *A. trachycaulum* and *H. jubatum* appears a much more logical interpretation. The formation of trivalents and quadrivalents possibly results from allosyndesis within one of the parents as suggested by STEBBINS *et al.* (1946) as one explanation for trivalents found in a hybrid between *A. trachycaulum* and *H. nodosum*. Their alternative interpretation, that of structural hybridity seems less likely in the present hybrids in view of the rarity of bridge fragments observed.

If the pairing is allosyndetic between chromosomes of *H. jubatum* and *A. trachycaulum* as seems very probable, then we can reasonably suggest that these two species have one set of chromosomes which are largely homologous. The genomic formulae AABB and AACC are therefore suggested for *A. trachycaulum* and *H. jubatum*, respectively.

SUMMARY

Both natural and controlled hybrids between *Agropyron trachycaulum* and *Hordeum jubatum* were studied. They are morphologically intermediate between the two parents, indistinguishable, sterile and very similar in the high incidence of unpaired

univalents and lagging chromosomes with resultant micronuclei. Eighty-nine percent of the cells observed contained at least 10 chromosomes associated in pairs or occasionally with one or two multivalents. They averaged 6.3 bivalents per cell. The evidence suggests that Hordeum and Agropyron have one genome in common.

These hybrids are comparable in every way to *Elymus macounii*. Our evidence from investigations on the meiotic chromosome behavior, comparative morphology and field observations of the parents and both natural and controlled hybrids demonstrates that at least some entities identifiable as *E. macounii* are simply F_1 sterile hybrids between *A. trachycaulum* and *H. jubatum*.

ACKNOWLEDGMENT

The authors wish to express their appreciation to DR. WESLEY KELLER who detected the hybrids and called our attention to them.

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STUDIES OF POLYPLOID SACCHAROMYCES. I. TETRAPLOID SEGREGATION[1]

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ASCI which exhibit tetraploid segregation are sometimes obtained from crosses between supposedly haploid strains of yeast (ROMAN, HAWTHORNE, and DOUGLAS 1951; LINDEGREN and LINDEGREN 1951). Their occurrence has led to the suggestion that a clone derived from a haploid ascospore may become a mixture of haploid and diploid cells and that crosses between such clones could give rise to triploid and tetraploid zygotes. The fact that diploidization does occur was demonstrated in clones which were originally haploid and which were selected for study because they had become heterogeneous for cell size (ROMAN and SANDS 1953). The diploid cells were found to be of two kinds, differing from each other in mating capacity and sporulating ability. One of these does not mate (except perhaps rarely, POMPER, DANIELS, and MCKEE 1954) with either of the standard mating types, **a** or **α**. It sporulates readily, however, to give asci in which, as a rule, two of the four spores are **a** and two are **α**. This diploid is therefore heterozygous for the mating-type alleles *a* and **α**; it arises as a result of mutation at this locus in some of the cells of the clone, and subsequent fusion of mutant and non-mutant cells. The diploid of the second kind cannot be induced to sporulate; it is capable of mating, however, and the proof that it is diploid comes from the results of crosses. Asci obtained from crosses with haploids exhibit segregations which are expected of triploids and which indicate that the diploid parent was homozygous at the mating-type locus (these data will be reported elsewhere). Confirmatory evidence is found in the results of crosses between the diploids themselves and is reported below. The types of segregation which are obtained indicate the similarity, with respect to chromosomal pairing and distribution in meiosis, between yeast and other organisms in which polyploidy has been studied.

MATERIALS AND METHODS

The origin of the diploid strains used in this investigation was described by ROMAN and SANDS (1953). The characters employed in the crosses were the following: the mating types **a** and **α**, rapid vs. slow galactose fermentation, and flaky vs. non-flaky dispersion in liquid medium. The corresponding allelic symbols are *a* and *α*, *G* and *g*, and *F* and *f*. Rapid fermentation and the flaky condition are dominant. Since at least three loci are known to be involved in galactose utilization, it should be mentioned that we are dealing here with the G_2-locus.

The LINDEGREN techniques for mating (1943) and for sporulation (1944) were

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utilized, according to a schedule which ran typically as follows. The diploids were crossed by placing inocula of each parent into 2 cc of a medium containing 1% yeast extract, 2% peptone, and 2% glucose. The next day, either the mating mixture was transferred to presporulation medium or a single zygote was isolated with a micromanipulator and transferred to a nutrient-agar (the above medium plus 1.5% agar) droplet; after two or three days, the colony obtained from the zygote was streaked on a nutrient-agar slant and after two or three days growth on the slant a sample was transferred to presporulation medium. In either case, the cells were grown on the presporulation slant for two or three days and were then placed on an acidified gypsum slant. Sporulation usually occurred within a week.

Only four-spored asci were selected for dissection. Each of the spores was placed on a nutrient-agar droplet. After two or three days, the colony which was produced from the spore was transferred to a nutrient-agar slant and after three or four days of growth on the slant, each segregant was tested for mating type, for its ability to ferment galactose, and for its flocculence in liquid medium. The slants were then refrigerated for later use.

The segregants were tested for mating type by mixing each with known **a** and α strains in 2 cc of the yeast extract-peptone-glucose medium; the mixture was examined the next day for copulation figures.

The ability to utilize galactose was tested by the Durham-tube technique in 8 cc of the above medium, except that 2% galactose was substituted for glucose. Clones which produced enough gas to fill the tubes within 48 hrs were classified as rapid fermenters; the slow fermenters did not produce visible amounts of gas for several days thereafter, if at all during the one to three-week period of test.

The flaky tests were conducted in Difco yeast nitrogen base medium to which 1% glucose was added. The standing tubes, containing 5 cc of this medium, were shaken 24 hrs after inoculation and the flocculence of flaky cultures was at this time in clear contrast to the uniform distribution of cells in nonflaky cultures.

In our interpretation of the results of crosses between the diploid strains, it is assumed that only one sporulation cycle occurred. An extra sporulation cycle could produce new zygotic combinations which could yield asci exhibiting segregations not expected from the original combination. The high proportion of unexpected segregations obtained by LINDEGREN and LINDEGREN (1951) with tetraploid material was attributed to uncontrolled sporulation. In our material, we have never seen asci on the presporulation slants and only very rarely on the nutrient-agar slants if the latter were stored under refrigeration. Sporulation is not negligible, however, if the slants are stored at room temperature.

EXPECTATIONS FROM TETRAPLOIDS

The types of segregation expected in asci from tetraploid cells are shown in figure 1, for a hypothetical locus represented by the alleles $+ + - -$. It is assumed that two of the four homologues go to each pole in meiosis, an assumption which has its exceptions as will be seen later. The chromosomes are diagrammed as bivalents, in chromatid condition; multivalent pairing would result in the same segregational types.

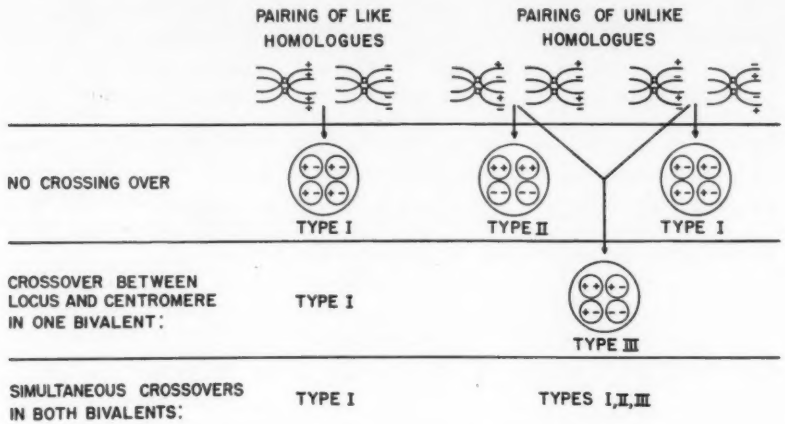

FIGURE 1.—Principal types of asci expected from tetraploid cells of genotype + + − − (see text).

In the absence of crossing over between the centromere and the locus, two types of segregation are expected. In type I, all four spores are heterozygous; in type II, two of the spores are homozygous for one allele and two are homozygous for the other. A crossover in this region between unlike homologues would result in a third type of segregation (III), in which one spore is homozygous for one allele, one is homozygous for the other, and two are heterozygous.

The phenotypic segregation for mating type, since it is possible to identify the heterozygous condition, is 4:0 for type I, 2:2 for type II, and 1:2:1 for type III. Rapid galactose fermentation and the flaky character are both dominant (we could not by our methods distinguish between the heterozygote and the homozygous dominant) and the phenotypic segregation for these two characters is 4:0, 2:2, and 3:1, respectively, for types I, II, and III.

RESULTS OBTAINED FROM TETRAPLOIDS

Table 1 summarizes the segregations obtained from tetraploids of composition *a a α α G G g g F F f f*. The segregations are tabulated separately for each locus. (Trisomic studies now underway indicate that the three loci are on different chromosomes.) The high frequency of type III segregation is evidence of considerable crossing over between each of the loci and its centromere, a point which will be returned to later.

A number of the segregants were subjected to further genetic test for the purpose of affirming their genotypes in the light of the expectations proposed above. The asci from which these segregants were taken, and the pattern of segregation in these asci, are given in table 2. In five of the asci, all four segregants were non-maters. These were induced to sporulate and, as would be expected if they were *a α*, the asci which were obtained exhibited segregation for the two mating types (table 3). The

TABLE 1

The distribution of the three types of asci, classified with respect to segregation for mating type, galactose utilization, and flaky, obtained from a a $\alpha\alpha$ G G g g F F f f *cells*

Cross	Genotypes of diploid parents	Mating type			Galactose utilization			Flaky		
		I	II	III	I	II	III	I	II	III
260L-I × 4L-I	*a a G G f f* × $\alpha\alpha$ *g g F F*	0	1	2	0	0	3	1	0	2
260L-I × 4L-II	*a a G G f f* × $\alpha\alpha$ *g g F F*	12	0	11	12	2	9	10	5	8
260L-I × 4L-III	*a a G G f f* × $\alpha\alpha$ *g g F F*	1	0	4	2	0	3	2	1	2
260L-V × 4L-I	*a a G G f f* × $\alpha\alpha$ *g g F F*	2	0	1	1	1	1	2	0	1
260L-V × 4L-II	*a a G G f f* × $\alpha\alpha$ *g g F F*	2	0	2	1	1	2	3	1	0
260L-V × 4L-III	*a a G G f f* × $\alpha\alpha$ *g g F F*	2	1	1	1	0	3	3	1	0
21L-41 × 1L-II	*a a g g f f* × $\alpha\alpha$ *G G F F*	6	2	2	5	0	5	3	2	5
21L-7 × 1L-II	*a a g g f f* × $\alpha\alpha$ *G G F F*	5	1	3	3	1	5	2	2	5
		30	5	26	25	5	31	26	12	23

TABLE 2

Asci from which segregants were selected for further genetic test. The phenotypes of the segregants are indicated as follows: n = non-mater; a or α = segregant capable of mating and of the corresponding mating type; G or g = rapid or slow galactose utilization; F or f = flaky or non-flaky

Cross	Ascus	Spores				Type of segregation		
		A	B	C	D	MT	G	F
260L-V × 4L-I	304	n G F	n G F	n G f	n G F	I	I	III
	305	n G F	n G F	n G F	n g F	I	III	I
260L-V × 4L-II	307	n G F	n g F	n g F	n G F	I	II	I
260L-V × 4L-III	310	a g F	n G F	α G F	n G F	III	III	I
	317	n G F	n G F	n G F	n G F	I	I	I
	319	α G f	a G F	α g f	a G F	II	III	II
	320	n G F	n g F	n G F	n G F	I	III	I

segregations for galactose fermentation and for flaky were also those expected. In ascus 317, however, two of the segregants (C and D) produced asci in which no more than two of the four spores were viable. This type of segregation would be the consequence if, in meiosis of the tetraploid ascogenous cell from which 317 arose, three of the four homologues were distributed to two of the spores (A and B) and the fourth homologue to the other two (C and D). Segregants C and D would thus be monosomic (for a chromosome not carrying one of the three loci) and would yield *n* and *n*-1 spores, the latter two being inviable. Genetic evidence for this type of meiotic distribution will be discussed below.

The six other segregants which were examined further—310A, 310C, 319A, 319B, 319C, and 319D—were all capable of mating and could not be induced to sporulate, a characteristic of diploids homozygous for mating type in this material. Their genotypes were determined from the results of intercrosses (table 4). The cross 310A × 310C was expected to give the zygotic genotype *a a* $\alpha\alpha$ *F F f f* and either *G G g g* or

TABLE 3

The results of tests of segregants listed in table 2

Segregant	No. of asci	Phenotypic ratio in asci			Genotype of segregant
		a:α	G:g	F:f	
304A	2	2:2	2:2	2:2	*a α G g F f*
B	2	2:2	2:2	4:0	*a α G g F F*
C	2	2:2	2:2	0:4	*a α G g f f*
D	2	2:2	2:2	2:2	*a α G g F f*
305A	2	2:2	4:0	2:2	*a α G G F f*
B	2	2:2	2:2	2:2	*a α G g F f*
C	2	2:2	2:2	2:2	*a α G g F f*
D	2	2:2	0:4	2:2	*a α g g F f*
307A	2	2:2	4:0	2:2	*a α G G F f*
B	2	2:2	0:4	2:2	*a α g g F f*
C	2	2:2	0:4	2:2	*a α g g F f*
D	2	2:2	4:0	2:2	*a α G G F f*
317A	2	2:2	2:2	2:2	*a α G g F f*
B	2	2:2	2:2	2:2	*a α G g F f*
C	7*	1:1	1:1	1:1	*a α G g F f*
D	6†	1:1	0:2	1:1	*a α G g F f*
		1:1	1:1	2:0	
320A	2	2:2	4:0	2:2	*a α G G F f*
B	2	2:2	0:4	2:2	*a α g g F f*
C	3	2:2	2:2	2:2	*a α G g F f*
D	1	2:2	2:2	2:2	*a α G g F f*

* In four of the seven asci dissected, two of the four spores survived to produce clones. The segregation tabulated is for the survivors of one of these asci. Of the remaining three asci, in two only one spore survived, and in the other none survived.

† In five of these asci only two spores survived and in the sixth there was one survivor. The data are given for two asci with two survivors.

TABLE 4

The results of tests of segregants listed in table 2 (continued). The numerals I, II, and III designate, respectively, the 4:0, 2:2, and 3:1 segregations obtained from + + − − cells. Segregations indicating a + − − − (G g g g or F f f f) genotype are given as numerical ratios, 2:2 or 1:3

Crosses of segregants	Segregations from crosses									Genotypes of segregants
	Mating type			Galactose			Flaky			
310A × 310C	8I	2II	12III	8I	4II	10III	13I	3II	6III	310A *a a g g F f* 310C *α α G G F f*
310A × 319A	14I	0II	7III	21	2G:2g		18	2F:2f		319A *α α G g f f*
							3	1F:3f		
319B × 319C	8I	2II	7III*	16	2G:2g		6I	3II	9III	319B *a a G g F F* 319C *α α g g f f*
				2	1G:3g					
319D × 319C	7I	2II	11III	7I	2II	10III†	6I	2II	12III	319D *a a G G F F*

* Not including one ascus (408) in which segregation for mating type was 2n:2α.

† Not including one ascus (456) in which segregation for galactose was 1G:3g.

G g g g, depending on whether 310C was *G G* or *G g*. Cells of the first type should yield segregations of types I, II, and III for all three loci; those of the second should give no type I or III for galactose fermentation. The results indicate that 310A was *a a g g F f* and that 310C was *α α G G F f*.

In the cross 310A × 319A, it was again necessary to distinguish between the two possibilities, *G G g g* or *G g g g*, in a genotype which was otherwise expected to be *a a α α F f f f*, depending on whether 319A was *G G* or *G g*. The results show that 319A was *α α G g f f*, since there were no type I or III segregations for galactose utilization in 21 asci dissected. Of special interest, however, is the occurrence of 1:3 ratios for the flaky condition in the asci from this cross. This segregation is expected in + − − − genotypes (HALDANE 1930; ROMAN, HAWTHORNE, and DOUGLAS 1951) from the following sequence of events: crossing over between the locus and its centromere, the distribution of the two + − dyads to the same pole, and a metaphase orientation in the second division to produce one spore carrying both + alleles. The other three spores would then be homozygous for the − allele. The diagnostic test for this type of ascus, therefore, consists of affirming that the dominant segregant is homozygous for the dominant allele. Tests of the 1:3 asci encountered here and in subsequent crosses will be discussed below.

The results of the cross 319B × 319C, the latter presumed to be *α α g g f f*, indicate that 319B was *a a G g F F*, rather than *a a G G F F*, the other possibility. Here again 1:3 ratios were obtained, this time for galactose utilization. There was also one ascus (408) in which the segregation for mating type did not conform to type I, II, or III. This anomaly will be discussed in a separate section.

The identification of the genotypes of 319A and 319B, with respect to galactose utilization, makes it clear that 319D should be *G G* and thus that the cross 319D × 319C should give *a a α α G G g g F F f f* zygotes. The occurrence of segregations of types I, II, and III for each of the three loci agrees with expectation. There was one ascus (456), however, which exhibited a 1:3 segregation for galactose utilization; this case will be discussed with the other anomalies.

TESTS OF THE SEGREGANTS IN THE 1:3 ASCI

The dominant segregants of five asci which exhibited a 1:3 ratio were subjected to further genetic test. The phenotypic segregations in the five asci are given in table 5 and the results of the genetic tests of the segregants are given in table 6. Segregants 453C, 432C, and 1120A were non-maters and were induced to sporulate. Segregants 425D and 499C were capable of mating and were crossed with the diploid recessive strain 319C. The results indicate that each of the segregants was homozygous for the dominant allele in question, as would be expected from the explanation proposed above for the origin of the 1:3 asci; that is, 425D and 453C were *F F* and 432C, 1120A, and 499C were *G G*. (The anomalous segregation for mating type in ascus 528 will be discussed separately.)

THE EXCEPTIONAL ASCI

The three exceptional asci which were encountered during the course of this investigation will now be discussed. They are 408, 456, and 528; their origin and pattern

TABLE 5

Phenotypic segregations in five asci exhibiting 1:3 segregation for either rapid galactose utilization or flaky

Cross	Ascus	Phenotypes of segregants			
		A	B	C	D
310A × 319A	425	n G f	n g f	α g f	a G F
a a g g F f × *α α G g f f*	453	n g f	n G f	n G F	n g f
319B × 319C	432	n g f	n g F	n G F	n g F
a a G g F F × *α α g g f f*	1120	n G f	n g f	n g F	n g F
425D × 319C	499	α g F	a g F	a G F	α g F
a a G g F F × *α α g g f f*					

TABLE 6

Results of tests of dominant segregants of 1:3 asci. The segregations are listed as I, II, or III if they were derived from + + − − genotypes; otherwise the numerical ratios are given with the dominant phenotype first in order

Segregant	Crossed by	Ascus	Segregation type			Genotype of segregant
			MT	G	F	
425D	319C	499	II	1:3	I	*a a G g F F*
	α α g g f f	500	I	2:2	I	
		501	III	2:2	I	
453C	Sporulated directly	527	2:2	2:2	4:0	*a α G g F F*
		536	2:2	2:2	4:0	
		537	2:2	2:2	4:0	
432C	Sporulated directly	1136	2:2	4:0	2:2	*a α G G F f*
		1137	2:2	4:0	2:2	
		1138	2:2	4:0	2:2	
		1140	2:2	4:0	2:2	
1120A	Sporulated directly	1609	2:2	4:0	0:4	*a α G G f f*
		1610	2:2	4:0	0:4	
		1611	2:2	4:0	0:4	
		1613	2:2	4:0	0:4	
		1614	2:2	4:0	0:4	
499C	319C	528	2n:2α	III	2:2	*a a G G F f*
		529	I	III	2:2	
		530	I	I	2:2	
		531	II	II	2:2	
		532	I	III	2:2	
		533	II	I	2:2	

of segregation are given in table 7. In asci 408 and 528, two of the segregants were incapable of mating and the other two were both of mating-type **α**. This type of segregation would be expected as a consequence of the unequal meiotic distribution of the chromosomes carrying the mating-type locus, so that two of the spores would be trisomic for this chromosome and *a a α*, and two would be monosomic, and *α*. In ascus 456, only one of the segregants was a rapid fermenter of galactose.

TABLE 7

Phenotypic segregation in exceptional asci 408, 456, 528

Cross	Ascus	Phenotype of segregants			
		A	B	C	D
319B × 319C *aa Gg FF* × *αα gg ff*	408	α G F	n G F	α g F	n g f
319D × 319C *aa GG FF* × *αα gg ff*	456	n g F	n g F	n g F	n G f
499C × 319C *aa GG Ff* × *αα gg ff*	528	α G F	n G f	α g f	n G F

In ascus 528, the genotypes of all four segregants could be established. Segregants 528A and 528C, both of which were capable of mating, were crossed with the diploids 21L-41 (*a a g g f f*) and 319D (*a a G G F F*), respectively, to test the assumption that these segregants carried a single α allele and were therefore monosomic for the mating-type chromosome. Segregants 528B and 528D, which were non-maters and presumably *a a α*, were induced to sporulate directly. The results obtained for the four segregants, and their confirmed genotypes, are given in table 8. The segregations for mating type are those expected from hybrids of genotype *a a α* and indicate that 528A and 528C were monosomic and that 528B and 528D were trisomic for the mating-type chromosome. The segregations for galactose fermentation and flakiness are also those expected.

In ascus 408, the segregants which were incapable of mating, 408B and 408D, could not be induced to sporulate and their genotypes remain unknown. However, 408A and 408C were capable of mating and were crossed, respectively, with the diploids 310A (*a a g g F f*) and 306B (*a a G G F f*); the segregations which were obtained (table 8) indicate that these two segregants, like 528A and 528C, were monosomic for the mating-type chromosome. The failure of 408B and 408D to sporulate suggests the possibility that these two segregants may have been aneuploid to a greater extent than were 528B and 528D.

Ascus 456 requires a more elaborate explanation. The 1:3 segregation for rapid galactose fermentation suggests that the ascogenous cell from which 456 arose had the genotype *G g g g*, rather than the *G G g g* genotype that was expected from the parentage. The change in genotype could have arisen as a result of mutation of *G* to *g*, either in the 319D parent or in the ascogenous cell. It could also have arisen as a consequence of an extra cycle of sporulation; if, for example, the expected tetraploid had sporulated on the presporulation slant to produce *G g* and *g g* cells, the fusion of these would result in a tetraploid of genotype *G g g g*. Our evidence does not permit us to distinguish between these possibilities.

The further analysis of the segregants of ascus 456 indicated an additional complication, this time in the distribution of the chromosomes carrying the locus for galactose fermentation. Each of the four segregants was a non-mater and was induced to sporulate. Segregants 456A, 456B, and 456C, which were themselves slow fermenters, did not produce rapid fermenters among their progeny (table 9). Segregant

TABLE 8

The results of tests of asci 408 and 528. See table 6 for distinction between segregations expressed by I, II, or III and those expressed as numerical ratios

Segregant	Crossed by	Ascus no.	Segregation type			Genotype of segregant
			MT	G	F	
408A	310A	497	2a:2α	2:2	II	*α G g F f*
	a a g g F f	503	2n:2a	2:2	I	
		504	2n:2a	2:2	I	
		505	2n:2a	2:2	III	
		506	1n:2a:1α	2:2	I	
408C	306B	492	3a:1α	II	II	*α g g F f*
	a a G G F f	493	1n:2a:1α	III	I	
		494	1n:2a:1α	III	I	
		495	1n:2a:1α	I	II	
528A	21L-41	1146	2n:2a	2:2	1:3	*α G g F f*
	a a g g f f	1147	2a:2α	2:2	2:2	
		1148	2n:2a	2:2	2:2	
		1149	1n:2a:1α	1:3	2:2	
		1150	2n:2a	2:2	1:3	
528B	Sporulated directly	1189	1n:2a:1α	4:0	0:4	*a a α G G f f*
		1190	2n:2a	4:0	0:4	
		1191	2n:2a	4:0	0:4	
		1192	1n:2a:1α	4:0	0:4	
528C	319D	1208	3a:1α	III	II	*α g g f f*
	a a G G F F	1209	2n:2a	I	II	
		1210	1n:2a:1α	III	III	
		1211	1n:2a:1α	I	I	
		1212	1n:2a:1α	I	I	
		1213	1n:2a:1α	I	III	
528D	Sporulated directly	1183	2a:2α	2:2	2:2	*a a α G g F f*
		1184	3a:1α	2:2	2:2	
		1185	2n:2a	2:2	2:2	
		1186	2n:2a	2:2	2:2	
		1188	2n:2a	2:2	2:2	

456D, a rapid fermenter, produced both rapid and slow fermenters, and the 3:1 ratios for rapid fermentation suggest that 456D was either *G G g g* or *G G g*. (The unexpected mating-type segregation in ascus 540 was not investigated further.) If the 3:1 segregations (in asci 538, 539, 540) arose from a *G G g g* genotype, two of the three rapid fermenters should be *G g* and the third should be *G G*. If the genotype were *G G g*, the three rapid fermenters should be *G G*, *G g*, and *G*, respectively, or, rarely, *G G*, *G*, *G*. Five of the six rapid fermenters in asci 538 and 539 were tested by crossing each with a rapid fermenter and a slow fermenter, and observing the segregations in the next spore generation. The results may be summarized as follows: 538D and 539A were found to be *G g*; 538B, 539B, and 539C were found to be *G*. Segregant 456D was therefore *G G g*.

Since 456D was trisomic for the chromosome carrying the galactose locus, it follows that one of the other segregants in 456 should also be trisomic and that two

TABLE 9

Results of genetic tests of ascus 456. The four-spored asci obtained from 456B and 456C gave only one or two survivors per ascus

Segregant	Phenotype	Ascus no.	Segregation type			Proposed genotype of segregant
			a:α	G:g	F:f	
456A	n g F	610	2:2	0:4	4:0	*a α g g g F F*
		611	2:2	0:4	4:0	
		612	2:2	0:4	4:0	
		613	2:2	0:4	4:0	
		614	2:2	0:4	4:0	
456B	n g F	617	1:1	0:2	1:1	*a α g F f*
		618	1:1	0:2	1:1	
		629	1:1	0:2	1:1	
		641	1:1	0:2	1:1	
		642	1:0	0:1	1:0	
456C	n g F	627	1:1	0:2	0:2	*a α g F f*
		628	1:1	0:2	1:1	
456D	n G f	538	2:2	3:1	0:4	*a α G G g f f*
		539	2:2	3:1	0:4	
		540	1n:2a:1α	3:1	0:4	
		634	2:2	2:2	0:4	
		635	2:2	2:2	0:4	
		1960	2:2	3:1	0:4	

of the segregants should be monosomic for this chromosome. The evidence presented in table 9 suggests that 456B and 456C were monosomic since each produced asci in which no more than two of the four spores were viable. It is therefore proposed that 456A was trisomic and *g g g*. We can thus account for ascus 456 as the result of the following sequence of events: 1) the production of an ascogenous cell of genotype *G g g g*, either as a consequence of mutation or an extra sporulation cycle; 2) the occurrence of a crossover between *G* and the centromere; and 3) the distribution of three chromosomes to one pole and one to the other.

THE USE OF TETRAPLOID SEGREGATION TO ASCERTAIN GENE-CENTROMERE LINKAGE

Since the type III segregation requires a crossover between a locus and the centromere, it should be possible to ascertain the amount of recombination in this region if the frequency of type III segregation is known. A precise relationship between this frequency and the frequency of recombination is difficult to establish, however, in the absence of cytological information on the way in which the chromosomes associate and disjoin in meiosis. The problem of measuring linkage in tetraploids has been discussed by MATHER (1936).

If it is assumed that the four homologues pair as bivalents, without preference, the frequency of type III asci can be related to the frequency of second-division segregation on the basis of the following considerations. Pairing between genetically unlike homologues should occur twice as often as pairing between like homologues (fig. 1). The latter leads only to type I segregation and crossing over in the region in

question would not be detected. A crossover in one of the bivalents of unlike homologues would result in second-division segregation for the chromatids of this bivalent and, therefore, in a type III segregation. Simultaneous crossovers in both bivalents would lead to segregations of types I, II, and III, in the proportion 1:1:2 respectively. Thus when pairing is between unlike homologues the frequency of type III segregation is related to the frequency x of second-division segregation by the equation:

$$f(\text{III}) = 2x(1 - x) + \tfrac{1}{2}x^2 = 2x - \tfrac{3}{2}x^2$$

This equation is adapted from the equation for segregation in unordered asci obtained from diploids (PERKINS 1949; WHITEHOUSE 1949). Taking the contribution from pairing of like homologues into account, the frequencies of the three ascus types may be calculated from the following:

$$f(\text{I}) = \tfrac{1}{3} + \tfrac{2}{3}[\tfrac{1}{2}(1 - x)^2 + \tfrac{1}{4}x^2] = (4 - 4x + 3x^2)/6$$

$$f(\text{II}) = \tfrac{2}{3}[\tfrac{1}{2}(1 - x)^2 + \tfrac{1}{4}x^2] = (2 - 4x + 3x^2)/6$$

$$f(\text{III}) = \tfrac{2}{3}[2x(1 - x) + \tfrac{1}{2}x^2] = (4x - 3x^2)/3$$

The frequencies are given in table 10 for various values of x. The table includes values of x as high as 100%; however, those in excess of 66.7% are unlikely unless interference extends over relatively long genetic distances in yeast. When x is small enough so that the effects of double crossing over may be disregarded without serious error, the frequency of crossing over is $x/2$. The correction to be applied for higher values of x requires information, at present unavailable for yeast, on the extent of interference (see BARRATT *et al.* 1954).

TABLE 10

Relation of frequency of second-division segregation (x) *to frequencies of segregation types I, II, and III expected from bivalent pairing (second, third, and fourth columns) or from tetravalent pairing followed by random disjunction of centromeres (last three columns)*

x (%)	From bivalent pairing			From tetravalent pairing		
	I	II	III	I	II	III
	%	%	%	%	%	%
0	66.7	33.3	0	66.7	33.3	0
10	60.5	27.2	12.3	60.4	29.3	10.3
20	55.3	22.0	22.7	54.9	26.0	19.1
30	51.1	17.9	31.0	50.2	23.5	26.3
33.3	50.0	16.7	33.3	48.8	22.8	28.4
40	48.0	14.7	37.3	46.2	21.8	32.0
50	45.8	12.5	41.7	43.1	20.8	36.1
60	44.7	11.4	44.0	40.7	20.7	38.7
66.7	44.4	11.1	44.4	39.5	21.0	39.5
70	44.5	11.2	44.3	39.1	21.3	39.7
80	45.3	12.0	42.7	38.2	22.7	39.1
90	47.2	13.9	39.0	38.2	24.8	37.0
100	50.0	16.7	33.3	38.9	27.8	33.3

It is, however, obvious from the results of segregation that pairing is not exclusively bivalent. If it were, we would not expect 1:3 segregations nor unequal distributions of chromosomes as revealed in asci 408, 456, 528, and perhaps in 317. It will be recalled that the 1:3 segregation is the result of a crossover between unlike homologues in $+ - - -$ cells, followed by the distribution of these two chromosomes to the same pole. If, after multivalent pairing, which we will assume to be tetravalent, disjunction of the centromeres, two to each pole, is random, the two crossover chromosomes should move to the same pole in one third of the cells in which crossing over has occurred, and the 1:3 segregation should be obtained from only half of these. Thus, no more than one sixth of the asci in which tetravalent pairing has occurred should exhibit a 1:3 segregation for a specific character. The fact that we have observed three 1:3 segregations for galactose utilization in 47 asci and three for the flaky character in 27 asci suggests that tetravalent pairing occurs with substantial frequency.

It would therefore be useful to know what effect tetravalent pairing would have on the frequencies of segregation types I, II, and III. It will be assumed that the centromeres disjoin at random, two to each pole, and that the segment between locus and centromere pairs as a unit. The first assumption may not be valid, for it is possible that if two of the four homologues engage in crossing over in the region between locus and centromere, that these two would have a tendency to disjoin preferentially. If this tendency were pronounced, the expectation from tetravalent pairing would approach that from bivalent pairing. The assumption is made, however, for the purposes of calculating a set of frequencies which is alternative to the set obtained from bivalent pairing, with the understanding that the correct frequencies may fall between the two sets. The second assumption, which presupposes that the chromatids do not exchange pairing partners, may be in error for relatively long distances; we assume that it is likely to hold for shorter distances, i.e., for those in which linkage between gene and centromere is demonstrable. On these assumptions, the frequencies of the segregational types are related to the frequency of second-division segregation as follows:

$$f(\mathrm{I}) = \tfrac{2}{9} + \tfrac{2}{3}[\tfrac{2}{3}(1-x)^2 + \tfrac{1}{6}\{2x(1-x)\} + \tfrac{1}{4}x^2] = (12 - 12x + 7x^2)/18$$

$$f(\mathrm{II}) = \tfrac{1}{9} + \tfrac{2}{3}[\tfrac{1}{3}(1-x)^2 + \tfrac{1}{4}x^2] = (6 - 8x + 7x^2)/18$$

$$f(\mathrm{III}) = \tfrac{2}{3}[\tfrac{5}{6}\{2x(1-x)\} + \tfrac{1}{2}x^2] = (10x - 7x^2)/9$$

The frequencies of the three types of segregation for different values of x are given in table 10. Expectations from the two modes of pairing are quite similar for small values of x. As x increases, the effect of tetravalent pairing becomes apparent in the relatively higher frequencies of the type II segregation, at the expense of types I and III.

The segregations from $+ + - -$ hybrids are summarized in table 11, for each of the three loci. Theoretical distributions, expected from bivalent and tetravalent pairing, are also given; these are calculated from the frequencies in table 10, for selected values of x. The G_2 and F loci do not exhibit linkage with the centromere in diploid segregation (HAWTHORNE 1955, and personal communication) and the value

TABLE 11

Summary of crosses giving type I, II, or III segregations. The theoretical distributions, for bivalent and tetravalent pairing, are given for selected values of x *(see text). Below each theoretical distribution is given, in parentheses, the corresponding chi-square value and probability*

Cross	Genotypes	Segregation types								
		MT			G			F		
		I	II	III	I	II	III	I	II	III
260L × 4L	*a a G G f f* × *α α g g F F*	19	2	21	17	4	21	21	8	13
310A × 310C	*a a g g F f* × *α α G G F f*	8	2	12	8	4	10	13	3	6
319D × 319C	*a a G G F F* × *α α g g f f*	7	2	11	7	2	10	6	2	12
21L × 1L	*a a g g f f* × *α α G G F F*	11	3	5	8	1	10	5	4	10
260L × 13L	*a a G G f f* × *α α g g f f*	4	1	5	7	2	1	—	—	—
425D × 319C	*a a G g F F* × *α α g g f f*	1	1	1	—	—	—	3	0	0
499C × 319C	*a a G G F f* × *α α g g f f*	3	2	0	2	1	3	—	—	—
319B × 319C	*a a G g F F* × *α α g g f f*	8	2	7	—	—	—	6	3	9
319D × 528C	*a a G G F F* × *α g g f f*	—	—	—	4	0	2	2	2	2
310A × 408A	*a a g g F f* × *α G g F f*	—	—	—	—	—	—	3	1	1
21L × 13L	*a a g g f f* × *α α g g f f*	6	1	2	—	—	—	—	—	—
310A × 319A	*a a g g F f* × *α α G g f f*	14	0	7	—	—	—	—	—	—
		81	16	71	53	14	57	59	23	53
Frequencies (%)		48.2	9.5	42.3	42.7	11.3	46.0	43.7	17.0	39.3
Theoretical distributions:										
For bivalent pairing	$x = 50\%$	77	21	70						
		(1.4; P = .5)								
	$x = 66.7\%$				55	14	55	60	15	60
					(.15; P = .9)			(5.1; P = .05–.1)		
For tetravalent pairing	$x = 50\%$	72	35	61						
		(13.1; P = <.01)								
	$x = 66.7\%$				49	26	49	53	28	53
					(7.2; P = <.05)			(1.6; P = .3 – .5)		

of x for these is taken as 66.7%. For the mating-type locus, the value of 50% is chosen since LINDEGREN (1949) and HAWTHORNE (1955) give frequencies of second-division segregation of 47% and 53%, respectively, for this locus. The chi-square values indicate that the results obtained for the G_2 and mating-type loci are in good agreement with the hypothesis of bivalent pairing, and are not expected from tetravalent pairing, whereas the reverse is true for the F locus. We interpret this to mean that while both types of pairing occur, bivalent pairing is more frequent for the chromosomes carrying the G_2 and mating-type loci and less frequent for the chromosome of the F locus. Excellent agreement is obtained with the observed results for the F locus if each of the two types of pairing is assumed to occur half the time.

POLYPLOIDY AND THE PROBLEM OF THE OCCURRENCE OF IRREGULAR RATIOS

When a cross is made between two clones, one carrying the gene + and the other the recessive allele −, and both clones have been derived from haploid ascospores, it is expected that the four-spored asci which are produced will segregate 2+:2−. However, "irregular" ratios—4:0, 3:1, 1:3, and 0:4—have also been obtained from such crosses and with frequencies which would seem to preclude conventional gene mutation as a likely explanation. Other mechanisms have therefore been suggested. LINDEGREN (1949, 1953) has proposed gene conversion, a special mutation hypothesis which assumes the alteration of a gene by its allele, in the heterozygous condition. WINGE and ROBERTS (1950), having observed that asci with more than four spores sometimes occur, have inferred from this that in some asci the four spores may be the survivors of an originally larger number and thus that some of the meiotic products have been lost. In some aberrant asci, it has been found that one of the spores is diploid whereas the other three are haploid. This has led to the hypothesis of "post-meiotic nuclear fusion" (ROMAN and SANDS 1953) which presupposes an extra mitotic division in the ascus with the subsequent fusion of haploid nuclei. WINGE and ROBERTS (1954) have obtained similar genetic evidence in homothallic as well as in heterothallic yeast, and cytological evidence of supernumerary mitoses and binucleate spores. Finally, polyploidy has been shown to contribute to the production of asci with irregular ratios (LINDEGREN and LINDEGREN 1951; ROMAN, DOUGLAS, and HAWTHORNE 1951; ROMAN and SANDS 1953; LEUPOLD and HOTTINGUER 1954). It thus seems clear that there are a number of reasons for irregular ratios when 2:2 segregation is expected.

In what follows we will restrict ourselves to a discussion of the consequences of polyploidy, which has been a recurring factor in the production of unexpected asci in the heterothallic material at our disposal. When a colony is obtained from a haploid spore on an agar droplet and is transferred either to 5 cc of broth or to a nutrient-agar slant, the resulting crop as a rule consists chiefly of haploid cells. If the clone is then subcultured in serial transfer, it frequently happens that after a number of transfers a subclone is obtained which consists largely or wholly of diploid cells. A clone which was originally haploid may thus be converted to a clone which is diploid; the diploid cells may be heterozygous or homozygous for the mating-type alleles, or both may be present in the same clone.

Suppose, therefore, that a cross is made between two clones originally of genotype $a+$ and $\alpha-$, respectively. In addition to the $a\ \alpha+\ -$ zygotes which are expected, polyploid zygotes may also occur and the types and frequencies of these would depend on the extent to which each of the parental clones consists of diploid cells homozygous for the mating-type alleles. Diploidization in only the a parent would lead to triploids of genotype $a\ a\ \alpha+\ +\ -$; if it occurred only in the α parent, $a\ \alpha\ \alpha+\ -\ -$ triploids would be expected. If diploid homozygotes were present in both parents, the tetraploid $a\ a\ \alpha\ \alpha+\ +\ -\ -$ could also be obtained.

Mating-type segregation is an excellent indicator for the detection of polyploid segregation. As table 11 shows, only about one out of 10 asci from tetraploid cells exhibits the $2\mathbf{a}:2\boldsymbol{\alpha}$ ratio which could be confused with diploid segregation. Charac-

teristic mating-type ratios serve to identify the triploid condition as well (see table 8) and here again the 2**a**:2**α** ascus is in the decided minority. The tetraploid condition and the triploid + + − can be identified also by the 4:0 and 3:1 segregations for the + phenotype. The frequency of 2:2 segregation is not expected to exceed ⅓ and would be less than this if crossing over occurs between the marker locus and the centromere, an event which is also necessary for the production of 3:1 segregation. The opportunity for detection of polyploid segregation depends on the frequency with which asci of polyploid origin arise in the cross and, in an individual ascus, on the number of segregating markers.

If, however, mating-type segregation is ignored as a criterion and triploid cells of genotype + − − are obtained in a cross, the detection of polyploidy is more difficult to achieve since this type of triploid is expected to yield chiefly asci with a 2:2 segregation. The 1:3 ratio is diagnostic; as can be seen from the results of segregation obtained from the comparable tetraploid condition, + − − −, which gave three 1:3 segregations for galactose utilization in 47 asci and three for the flaky character in 27 asci, the opportunity for the detection of triploids of this type would not be great unless a relatively large number of asci were dissected. Thus 2:2 segregation cannot in all circumstances be taken as evidence of diploid segregation unless triploidy has been excluded as a factor: this can be accomplished if several markers are used and the cross is made so that dominants are contributed by each of the two parents.

If segregants obtained from polyploids are used in crosses, either unwittingly or for further genetic test, the fact that aneuploid combinations may arise must also be taken into account in the interpretation of the results of crosses. Aneuploid spores are produced by tetraploids and, more commonly, by triploids (unpublished evidence). A cross between a haploid and an aneuploid of composition $2n\text{-}x$ would, for example, yield diploid segregation ratios for the markers located on the chromosomes present in disomic condition in the zygote and triploid ratios for the others. Moreover, an aneuploid clone can become a mixture of aneuploid and euploid cells, possibly as a consequence of mitotic nondisjunction and the competitive advantage enjoyed by the euploid; evidence is at hand that this happens in clones which are disomic for one chromosome and otherwise haploid. For example, in clones disomic for the chromosome carrying the G_2 locus and of genotype $G_2\ g_2$, haploid cells which have lost either G_2 or g_2 can be identified genetically. Such clones, although heterogeneous, are classified as fermenters; in crosses with nonfermenter haploids, they yield asci exhibiting 2:2, 1:3, and 0:4 ratios for galactose fermentation. The relative frequencies of the three types of asci depend of course on the balance existing between the three types of cells in the heterogeneous culture.

Thus polyploidy and its consequences may lead to the production of each of the four types of irregular asci. If polyploidy is excluded as a factor, either by appropriate genetic test of the segregants or by segregational evidence exhibited by the aberrant ascus itself, other mechanisms may be invoked. Some of these have been mentioned above; others may come to light as further information is obtained on the genetics of yeast.

SUMMARY

Evidence is presented to show that tetraploid segregation in yeast follows the pattern which could be expected of an orthodox meiosis. Tetraploids of genotype + + − − yield asci which exhibit phenotypic ratios of 4:0, 3:1, and 2:2; those of genotype + − − − yield 2:2 and 1:3 asci. The effect of multivalent pairing is seen in the occasional production of exceptional asci, which contain aneuploid spores.

Calculations are made relating the frequency of second-divison segregation to the frequencies of the three types of asci obtained from the + + − − tetraploid, thus providing a means of determining gene-centromere linkage. Two sets of calculations are given, one based on bivalent pairing, the other on tetravalent pairing. The observed frequencies of segregation for three loci suggest that both types of pairing occur.

The problem of the occurrence of irregular asci is discussed in terms of polyploidy and its consequences.

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